2003

Silica Supported Copper Complexes and their Biomimetic Activities

Jun Wang
College of William & Mary - Arts & Sciences

SILICA SUPPORTED COPPER COMPLEXES AND THEIR BIOMIMETIC ACTIVITIES

A Thesis

Presented to

The Faculty of the Department of Chemistry

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Science

---

by

Jun Wang

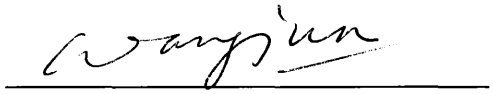
2003

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

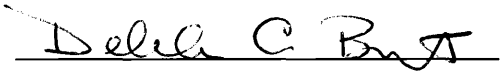
The requirements for the degree of

Master of Arts

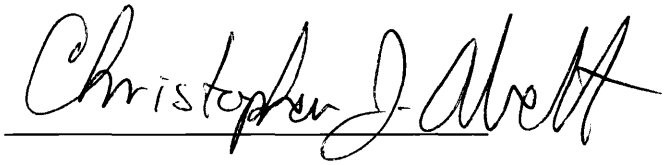


Author

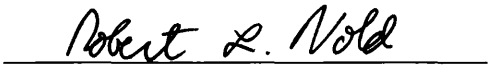
Approved, August 2003



Dr. Deborah C. Bebout



Dr. Christopher J. Abelt



Dr. Robert L. Vold

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The author would like to express her sincere appreciation to Dr. Bebout for her invaluable guidance and understanding during this research. The author is also indebted to Dr. Abelt and Dr. Vold for their contributions to this manuscript and careful instructions during this endeavor.

The author wishes to acknowledge the exceptional support of the Department of Chemistry and all her friends, especially the entire Bebout group, without which her research experience would not have been complete. In particular, thanks are given to the undergraduate students Somang Kim and Becky Monsess.

Finally, the author wishes to express her deepest appreciation to her parents for their very generous support. They provided her with encouragement and strength throughout her study.

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

BPA = bis[(2-pyridyl)methyl]amine

4-NBC = 4-nitrobenzyl chloride

TSPI = 3-(triethoxysilyl)propyl isocyanate

$BPAS_4$ = 4-nitrobenzyl-bis[(2-pyridyl)methyl] silylated amine

*i*bCO = sweet potato catechol oxidase

HC = hemocyanin

TYR = tyrosinase

CO = catechol oxidase or catecholase

DTBC = 3,5-di-*tert*-butylpyrocatechol

TCC = tetracholorocatechol

EBA = 1,6-bis[[bis(1-methyl-2-benzimidazolyl)methyl]amino]-*n*-hexane

L-55 = α,α'-bis[[bis(1-methyl-2-benzimidazolyl)methyl]amino]-*m*-xylene

L-66 = α,α'-bis[bis[2-(1-methyl-2-benzimidazolyl)ethyl]amino]-*m*-xylene

H-BPMP = 2,6-bis[(bis(2-pyridyl(methyl)amino)methyl)-4-methylphenol

BLC = beef liver catalase

$BPAN_4$ = 4-nitrobenzyl-bis[(2-pyridyl)methyl]amine

$BPAA_4$ = 4-aminobenzyl-bis[(2-pyridyl)methyl]amine

ABSTRACT

The coordination chemistry of copper is a subject of considerable importance in connection with the structures and active sites of copper-containing metalloproteins and enzymes. Many biomimetic metal complexes have been synthesized and their catechol oxidase and catalase activities investigated. In this research, one tridentate ligand was silylated, bound to copper(II) under basic conditions and attached to silica, providing a new heterogeneous catalyst.

The tridentate nitrogen ligand bis[(2-pyridyl)methyl]amine (BPA) was chosen for investigation of its potential as the ligand component of a heterogeneous copper catalyst. The conditions for derivitization of BPA for incorporation into a silica matrix were optimized, using 4-nitrobenzylchloride (4-NBC) addition and ammonium sulfide reduction strategies. The resulting amine was added to 3-(triethoxysilyl)propyl isocyanate (TSPI) to form the silylated urea 4-nitrobenzyl-bis[(2-pyridyl)methyl]silylated amine ($BPAS_4$).

The research focused on how the pH used for catalyst preparation, methods to prepare the catalyst, as well as the Cu(II) content of the catalyst affected catalytic activities. Base has been found to affect the structure of Cu(II) complexes, leading to different catalytic activities. The catechol oxidase and catalase activities of silicas modified at pH 8, 9, 10, 11, 12 and 13 with Cu and $BPAS_4$, were investigated and compared. The relationship between Cu(II) content and 3,5-di-*tert*-butylpyrocatechol (DTBC) oxidation rate, representative of catechol oxidase activity, at different pH was studied. To investigate the possible impact of hydroxide bridged binuclear copper centers as templates during silica modification, two methods were used to synthesize catalysts. In the first method, copper was present during silica modification with $BPAS_4$. In the second method, copper was added to the $BPAS_4$ modified silica. As expected, catalase activity was notably greater when the first method was used. Future studies will compare the catechol oxidase activity of catalysts prepared by both methods. Differences in catechol oxidase activity between different batches of $BPAS_4$ were noted and require further investigation. Additional studies conducted included investigation of the relationship between Cu(II) content and catechol oxidase activity as a function of pH and comparison of the homogeneous Cu·BPA catalyst to Cu·$BPAS_4$ modified silica.

These preliminary results encourage further studies. First, methods providing heterogeneous catalysts with reproducible properties must be developed. Complete characterization of the catalase and catechol oxidase activities of Cu·$BPAS_4$ modified silica will then be possible. Future studies may also investigate the ability of Cu·$BPAS_4$ modified silica to promote additional copper dependent biomimetic activities such as reactions promoted by dopamine β-monooxygenase and peptidylglycine α-amidating monooxygenase.

SILICA SUPPORTED COPPER COMPLEXES AND THEIR BIOMIMETIC ACTIVITIES

CHAPTER 1. INTRODUCTION

1.1. Overview of literature and research focus

Copper has been recognized as a biologically essential element for a long time.[1] Copper is a key cofactor in a diverse array of biological oxidation-reduction reactions. These reactions involve either outer-sphere electron transfer, as in the blue copper proteins and Cu_A site of cytochrome *c* oxidase and nitrous oxide reductase, or inner-sphere electron transfer in the binding, activation, and reduction of dioxygen, superoxide, nitrite and nitrous oxide.[2]

The structure and catalytic function of many enzymes containing copper ions at the active sites have been studied. These studies have permitted design of ligands that bind copper producing synthetic systems modeling the catalytic activity of enzymes. Common activities to screen new copper catalyst for include catechol oxidase, catecholase and catalase.[3-18]

New catalysts can be classified as homogeneous systems, which fully dissolve in solvent, or heterogeneous systems, which are attached to an inert solid support such as silica and remain phase separated during catalysis. Recycling of homogeneous catalysts is typically impractical, increasing their expense. Simple heterogeneous catalysts typically have a large proportion of active sites that are inaccessible, reducing their apparent activity. Recently, catalysts prepared by covalent attachment of appropriately modified ligands to inert substrates have been demonstrated to combine the important attributes of heterogeneous and homogeneous catalysts. [19-21]

The research described in this thesis focuses on the catalytic potential of copper when bound to a tridentate nitrogen ligand with a silylated tether that permitted attachment to silica. Variations in the conditions for silica modification were explored. Characterizations of modified silicas included copper loading, catechol oxidase activity and catalase activity. Comparisons with related systems are made.

1.2. Classification of the of copper centers in proteins

Copper centers in proteins have historically been divided into three classes based on the spectroscopic features of the active site.[22] The mononuclear type-1 copper centers, "blue copper centers", are found in electron-transfer proteins such as plastocyanin and azurin. Their deep blue color is caused by an intense Cys S-Cu (II) charge-transfer transition. A type-1 copper center has a strongly distorted tetrahedral coordination sphere with a difference of up to 22° from the ideal tetrahedron angle.[23] The mononuclear "non-blue copper centers" are classified as type-2 centers. They are found in oxidases, such as galactose oxidase, and in oxygenases, such as dopamine-β-monooxygenase. A type-2 copper center also appears in the dinuclear metal site of Cu, Zn-superoxide dismutase. A square-planar coordination sphere characterizes type-2 copper centers. In contrast to these mononuclear copper sites, the type-3 copper centers contain coupled binuclear copper sites and are described in section 1.3.

During the past fifteen years, additional classifications have been created for non-type 3 multinuclear copper centers. For example, proteins such as laccase, ascorbate oxidase, and ceruloplasmin contain trinuclear copper clusters, comprised of a type 2 and a type 3 center.[24-27] Examples of mixed-valent binuclear copper sites [3, 4, 28-32] include the Cu_A-Cu_B-$heme_A$ center (Cu_A is the copper in subunit II, Cu_B is most likely in subunit I,

and heme$_A$ is in subunit I) of cytochrome *c* oxidase,[3] and the binuclear Cu$_Z$ center (Z is the catalytic center and also one of two distinct copper centers) of nitrous oxide reductase.[4]

1.3. Proteins with type-3 copper centers

Proteins containing binuclear copper centers play important roles in biology, including dioxygen transport or activation, electron transfer, reduction of nitrogen oxides, and hydrolytic chemistry.[5] The typical structure of type-3 copper centers contain two copper ions that are linked by two oxygen atoms. Hemocyanins (HC), tyrosinases (TYR), and catechol oxidases or catecholases (CO) contain type-3 copper centers. HCs serve as oxygen carrier proteins, binding molecular oxygen reversibly.[2] HCs are divided into two classes depending on their biological source: the arthropodan and the molluscan. TYRs catalyze both the hydroxylation of phenols to *ortho*-diphenols (cresolase activity) and the two-electron oxidation of *ortho*-diphenols to *ortho*-quinones (catechol oxidase activity) by molecular oxygen. [2] COs are also known as *ortho*-diphenol oxidases. The official nomenclature of CO is 1, 2-benzenediol: oxygen oxidoreductase, indicating that dioxygen is the second substrate. COs catalyze the oxidation of a wide range of *ortho*-diphenols to *ortho*-quinones in the presence of oxygen[6], but do not exhibit cresolase activity (Figure 1.1).[7]

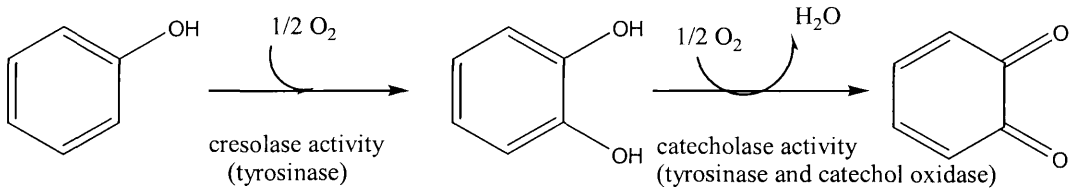


Figure 1.1. Reaction pathway of the oxygenation and oxidation catalyzed by TYR and CO. (Ref. 7)

The functional and structural differences between HC, TYR and CO could not be rationalized for a long time, as detailed structural information was available only for arthropodan HCs. These enzymes are all organized into seven different domains.[27] The two copper-binding regions show the highest conservation among all type-3 copper proteins. The region binding Cu_B (see Figure 1.2) is especially highly conserved, whereas the Cu_A binding region shows more sequence variety and has been held responsible for the differing function of HC, TYR, and CO.[8] The 3D structure of TYR is not yet known, but the recent structural data for a molluscan HC and a plant CO allow a deeper insight into the active site of type-3 copper proteins.

The crystal structure of the active site containing two copper ions of sweet potato catechol oxidase (ibCO) is shown in Figure 1.2.[8,9] Both copper atoms forming the metal center are coordinated by three histidines.[9,10] An unusual covalent thioether bond joins Cys92 to the Cu-coordinating His109 (Figure 1.2). The Cys-His thioether bridge has been found only in type-3 copper proteins but does not seem to be essential, as it is absent from arthropodan HC crystal structures.

In the *met* state (Cu (II), Cu (II)) of ibCO, the two cupric ions are at a distance of 2.9 Å. They are bridged by another atom, most likely a hydroxide ion, at a distance of about 1.8 Å from each cupric ion, so that each of them has a coordination number of four.[10] The coordination sphere of each copper atom is a trigonal pyramidal with His109 and His240 in the apical positions, respectively.

In the *deoxy* state, both copper atoms are in the +1 oxidation state. The copper-copper distance is 4.4 Å. The coordination number is 4 for Cu_A (three histidine ligands and a water molecule) and is 3 for Cu_B (three histidines ligands). The coordination sphere

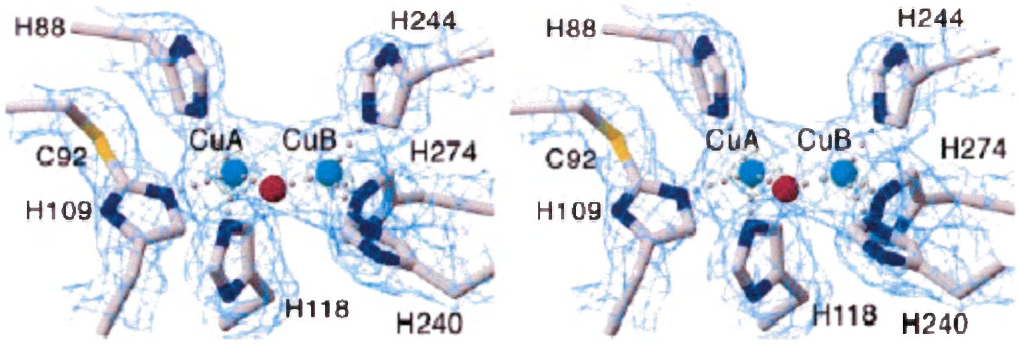


Figure 1.2: A sample of 2 |Fo| - |Fc|Φ_{alc} electron density, for the oxidized catalytic dinuclear copper site of sweet potato catechol oxidase. The electron density is shown contoured at 1.5 times the r.m.s. deviation of the map in cyan and contoured at 6 srms in dark green. Both copper sites, CuA on the left and CuB on the right, show a trigonal pyramidal coordination sphere formed by three histidine ligands and the bridging solvent molecule. The sulfur atom of Cys 92 does not ligate to the copper center but is covalently bound to the C_ε atom of His 109. (Ref. 9)

is distorted trigonal pyramidal for Cu_A and is square planar for Cu_B (the coordination site occupied by the bridging OH^- in the *met* state is vacant).

Though the structure of CO is known, the catalytic mechanism is still unclear. The catalytic mechanism of the related enzyme TYR was first studied in detail by Solomon and coworkers.[2] The currently accepted mechanism is shown in Figure 1.3.[7] The *oxy* state is proposed to be the required starting point for cresolase activity (right loop). A monophenol substrate bound to the *oxy* state becomes monooxygenated to *o*-diphenol. This diphenol subsequently binds to the copper centers of *met*TYR, to become oxidized and produce the *deoxy* copper state. Dioxygen reoxidizes the reduced copper center to the *oxy* state and closes the cycle. Catecholase activity (outer loop) is proposed to be possible starting from both the *oxy* and *met* states. Oxidation of a diphenol interacting with either state produces a pathway for their interconversion.

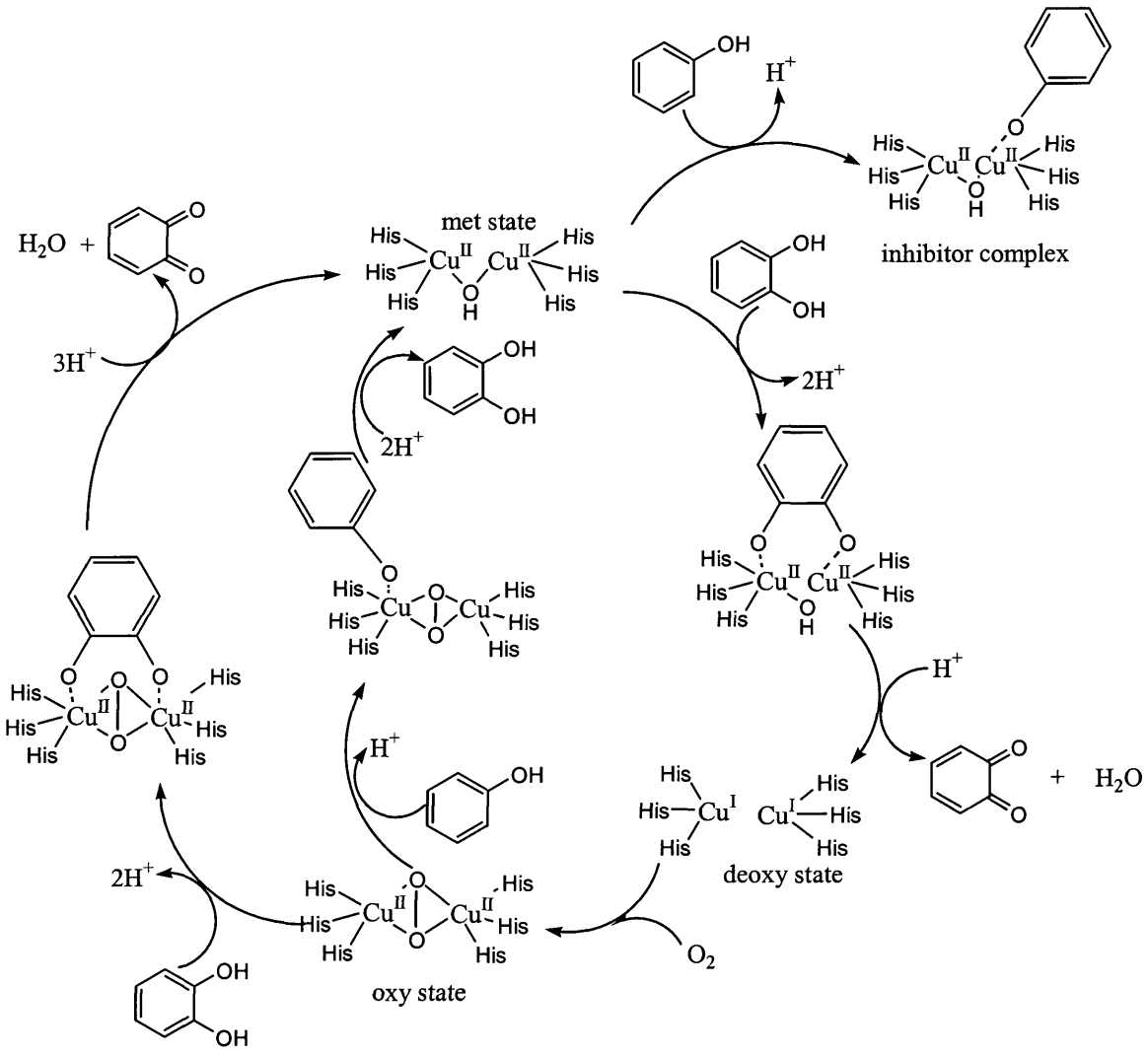


Figure 1.3: Mechanism of cresolase and catecholase activity of TYR and/or CO. It is developed on the basis of an initial proposal by Solomon and co-workers and including more recent results. (Ref: 2, 7)

1.4. Synthetic models of type-3 copper centers

Grenstead studied the metal-catalyzed (Mn^{2+}, Co^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+}) oxidation of 3,5-di-*tert*-butyl pyrocatechol (DTBC) by oxygen and oxidation of quinone by hydrogen peroxide.[11] Two possible mechanisms for the two electron oxidation of DTBC were proposed: (1) scavenging of aryl radicals by a polyvalent metal-ion catalyst that oxidizes them to quinones before side reactions can occur; (2) production of a very

reactive semiquinone radical as a one-electron intermediate, which rapidly loses a second electron. The second mechanism is commonly accepted. Two years later, Tsuji reported the smooth oxidative cleavage of the aromatic ring of a simple catechol with molecular oxygen activated by cuprous chloride at room temperature, the first successful model for pyrocatechase (Figure 1.4):[12]

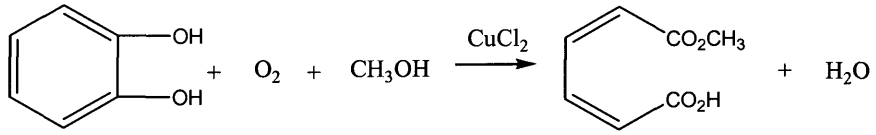


Figure 1.4: Oxidation of catechol by oxygen in methanol activated by cuprous chloride. (Ref. 12)

There are several characteristic features of this reaction. The product was the unexpected monomethyl ester. The diester or free acid was not obtained although the reaction was carried out with various concentrations of methanol. The addition of 1.5 molar equiv of methanol gave 72% yield of the mono ester. Higher concentrations of methanol were not favorable and the yield dropped to 42% with 20 molar equivalents of methanol. As the catalyst for the reaction, cuprous chloride seemed to be the best one, while other metal salts such as cupric chloride showed no activity.

Many other binuclear copper(II) ligand complexes have been synthesized and evaluated for catechol oxidase related activity during the past several decades.[13-15, 16-17, 19-21, 33-39] For example, Karlin and coworkers synthesized a well-defined binuclear copper complex containing a catechol ligand coordinated to and bridging the two Cu(II) ions of a phenoxo-bridged binuclear copper(II) complex.[33] The complex contained a bridging tetrachlorocatechol (TCC) between the two copper(II) ions with a metal-metal separation of 3.248 Å. An intramolecular two-electron-transfer reaction produced the *o*-quinone

product and regenerated the binuclear Cu(I) center. This was the first study to structurally characterize complex/substrate adducts in a catechol oxidase model.

Based on other researchers' work, Casella and coworkers synthesized a series of copper(II) complexes and compared their catechol oxidase activities.[16, 35-36] The investigated ligands included 1,6-bis[[bis(1-methyl-2-benzimidazolyl)methyl]amino]-n-hexane (EBA), α,α'-bis[[bis(1-methyl-2-benzimidazolyl)methyl]amino]-*m*-xylene (L-55) and α,α'-bis[bis[2-(1-methyl-2-benzimidazolyl)ethyl]amino]-*m*-xylene (L-66). These ligands were the first to permit demonstration of a true tyrosinase pathway in a catalytic catecholase reaction.[36] The activities of the dicopper(II) complexes of L-55, L-66, and EBA were compared in the catechol oxidase reaction. The relative rates for the fast first step involving electron transfer between a presumably bridging catechol anion and the dicopper(II) centers were found to be: $[Cu_2(L\text{-}66)]^{4+} > [Cu_2(L\text{-}55)]^{4+} > [Cu_2(EBA)]^{4+}$. This step was clearly dominated by the reduction potential of the Cu(II)/Cu(I) couple. Completion of the reaction involved oxygenation of the dicopper(I) species, binding of the catechol to the Cu_2O_2 intermediate, and electron transfer from the catechol anion to the dioxygen moiety. For $[Cu_2(L\text{-}66)]^{2+}$, the reaction with dioxygen was particularly slow, limiting the efficiency of the L-66 complex. In contrast, oxygenation of $[Cu_2(L\text{-}55)]^{2+}$ and $[Cu_2(EBA)]^{2+}$was relatively fast. The step limiting the overall catalytic process in these cases was the binding and reaction of catechol with the oxygenated species. Between the L-55 and EBA complexes, the former was the more effective catalyst because the ligand was better suited to maintain a folded structure, which facilitated binding of the bridging substrate. Furthermore, the EBA complex underwent an inhibition process at high catechol concentration that did not affect the L-55 complex.

Recently, Belle and coworkers used a series of ligands to investigate the correlation between electronic and pH effects on catechol oxidase activity. Derivatives of 2,6-bis[(bis(2-pyridylmethyl)amino)methyl]-4-methylphenol (H-BPMP) were used as copper ligands.[37] The pH-driven interconversion process was investigated. Substitution of the methyl group of H-BPMP (HL_{CH3}) by electron withdrawing (F or CF_3) or electron donating (OCH_3) groups afforded a series of dinucleating ligands (HL_{OH3}, HL_F, HL_{CF3}). In basic medium, new species were reversibly formed and identified as the bis hydroxo complexes except for the complex from HL_{CF3}, which was irreversibly transformed near pH 10. The pH–dependence of the catalytic abilities of these complexes was related to changes in the coordination sphere of the metal centers: only the μ-hydroxo complexes of HL_{OH3}, HL_F, HL_{CF3} exhibited catechol oxidase activity. The electronics of the R-substituents were associated with drastic effects on the catechol oxidase activity: the presence of an electron donating group on the ligand increased this activity; the reverse effect was observed with an electron withdrawing group.

Though previous research has focused mostly on homogeneous copper catalysts, some heterogeneous catalysts have also been studied. For example, Bowman and coworkers studied the catechol oxidase and catalase activities of catalysts based on $[Cu(1, 10\text{-phen})_2]^+$ (phen = phenanthroline) complexes immobilized on silica.[19-21] The dimeric forms of the silica supported copper(I) species were far more active for H_2O_2 decomposition and DTBC oxidation than the monemeric catalyst. The relevant oxidized form of catalyst, $[Cu(1, 10\text{-phen})]^{2+}$ was also investigated for DTBC oxidation and H_2O degradation. $[Cu(1, 10\text{-phen})_2(OH)_2]^{2+}$ was apparently the active species as a 1:1 Cu:ligand ratio and higher pH enhanced catalysis.

1.5.Catalases and H_2O_2 in living organisms

A variety of metalloenzymes catalyze H_2O_2 decomposition,[21] most of which are heme enzymes. Hydrogen peroxide, a by-product of various oxidases and superoxide dismutases, is not only toxic by itself, but can also decompose to form the even more reactive hydroxyl radical. This radical is probably the most deleterious of the activated intermediates of oxygen, reacting with DNA, proteins and lipids in its proximity.[40]

For the detoxification of H_2O_2, and probably also for maintaining its regulatory function, nature has developed a family of highly effective enzymes, catalases, which dismutate H_2O_2 according to the equation: [41]

$$2\,H_2O_2 \xrightarrow{\text{catalase}} O_2 + H_2O$$

Thenard discovered H_2O_2 in 1811 and predicted its decomposition in living tissues was performed by a special substance. In 1863, Schonbein identified a certain kind of "ferment" that was later named "catalase" by Loew in 1901. The active site of catalase was demonstrated to contain iron 22 years later.[42] In 1927, Wieland offered a simple explanation for the catalase function in cells, suggesting H_2O_2 was acting as a hydrogen donor for a hypothetical catalyst, which in hydrogenated form reduced another molecule of H_2O_2 to water. Nine years later, Stern demonstrated that in all then known catalases, protoporphyrin IX was the active group, and the first crystals of beef liver catalase (BLC) were obtained a year later. The first prokaryotic catalase was obtained from *Micrococcus luteus* by Herbert and Pinsent. The first electron density maps were obtained by Vainshtein et al. for a catalase from a lower eukaryote at 3.5 Å. The sequences of about 80 true catalases are currently available in public database and six three-dimensional structures of catalases are solved to high resolution. According to

structural and functional similarities, catalases can be divided into three subgroups: heme containing typical catalases, heme containing catalase-peroxidases, and non-heme managanese catalase.[43]

1.6.Copper(II) complexes mimicking catalase activity

It has been know for about one century that the decomposition of H_2O_2 is dramatically accelerated by many metal ions, among which Fe^{3+}/Fe^{2+} and Cu^{2+} have been investigated in depth. Disagreements over mechanistic details, especially the involvement radicals or peroxy complexes as intermediates have lasted for decades.[43, 45]

Sigel and coworkers studied the kinetics of the decomposition of H_2O_2 in acidic solution.[44] A mechanism involving peroxy complexes but no radicals were suggested:

$$H_2O_2 \overset{K_1}{\rightleftharpoons} HOO^- + H^+$$

$$[Cu(bipy)^{2+}] + HOO^- \overset{K_2}{\rightleftharpoons} [Cu(bipy)(OOH)]^+$$

$$[[Cu(bipy)(OOH)]^+ + H_2O_2 \overset{K_3}{\rightleftharpoons} [Cu(bipy)(OOH)(H_2O_2)]^+$$

$$[Cu(bipy)(OOH)(H_2O_2)]^+ \xrightarrow{k^*} [Cu(bipy)]^{2+} + O_2 + H_2O + OH^-$$

In this mechanism, rather than changing its oxidation state, Cu^{2+} plays the role of a catalyst template that forms a bridge linking the two molecules of H_2O_2 that undergoes oxidation and reduction.

In 1988, Luo and coworkers proposed a possible mechanism of H_2O_2 decomposition catalyzed by Cu^{2+} in alkaline solution.[45] It suggested that the observed yellow complex of copper and peroxide is an essential intermediate in the alkaline catalysis. Under the assumption that $Cu(OH)_3^{1-}$ and $Cu(OH)_4^{2-}$ were the only

nonnegligible species of copper hydroxide in the pH range 11-12, the following equilibria were proposed:

$$H_2O_2 + OH^- \rightleftharpoons HO_2^- + H_2O \quad (M_1)$$

$$Cu^{2+} + HO_2^- + (n+1)\,OH^- \rightleftharpoons O_2Cu(OH)_n^{n-} + H_2O \quad (M_2)$$

$$Cu^{2+} + H_2O_2 + (n+1)\,OH^- \rightleftharpoons HO_2Cu(OH)_n^{(n-1)} + H_2O \quad (M_3)$$

$$Cu^{2+} + 3OH^- \rightleftharpoons Cu(OH)_3^- \quad (M_4)$$

$$Cu^{2+} + 4OH^- \rightleftharpoons Cu(OH)_4^- \quad (M_5)$$

$$O_2Cu(OH)_2^{2-} \longrightarrow Cu(OH)_2^- + O_2^{\cdot -} \quad (M_6)$$

$$HO_2Cu(OH)_2^- \longrightarrow Cu(OH)_2^- + HO_2^{\cdot -} \quad (M_{6'})$$

$$Cu(OH)_2^- + O_2 + OH^- \longrightarrow Cu(OH)_3^- + O_2^{\cdot -} \quad (M_7)$$

$$Cu(OH)_2^- + H_2O_2 \longrightarrow Cu(OH)_3^- + OH^{\cdot} \quad (M_8)$$

$$Cu(OH)_2^- + O_2 + H_2O \longrightarrow Cu(OH)_3^- + OH^- + OH^{\cdot -} \quad (M_{8'})$$

$$OH^{\cdot} + H_2O_2 \longrightarrow H_2O + HO_2^{\cdot} \quad (M_9)$$

$$R + R \longrightarrow H_2O_2(HO_2^-) + O_2(+OH^-) \quad (M_{10})$$

A suitable combination of eq M_1-M_{10} yields the stoichiometry of the disproportionation of H_2O_2 in an alkaline Cu(II) solution:

$$5\,Cu^{2+} + 4\,H_2O_2 + 15\,OH^- \longrightarrow 5\,Cu(OH)_3^- + 2\,O_2 + 4\,H_2O$$

Seven years later, Bonomo and coworkers reported H_2O_2 interaction with copper(II) complexes of diamino-diamide type ligands, diastereoisomeric dipeptides, and tripeptides.[46] Their results supported Luo's hypothesis.

The stoichiometry and kinetics of the reduction of cupric complexes of the bidentate ligand 2, 9-*di*-methyl-1, 10-phenanthroline (DMP) by H_2O_2 have also been studied. Since the overall stoichiometry was determined with this system,[18] it can be used to monitor H_2O_2 decomposition. The mechanism suggested by Davies included the following six steps:

$$Cu(DMP)^{2+}(aq) + DMP \longrightarrow Cu(DMP)_2^{2+}(aq) \quad (1)$$

$$Cu(DMP)_2^{2+}(aq) \longrightarrow Cu(DMP)_2OH^{+}(aq) + H^{+}(aq) \quad (2)$$

$$H_2O_2(aq) \longrightarrow HO_2^{-}(aq) + H^{+}(aq) \quad (3)$$

$$Cu(DMP)_2^{2+}(aq) + HO_2^{-}(aq) \longrightarrow Cu^{I} + O_2^{-} + H^{+}(aq) \quad (4)$$

$$Cu(DMP)_2OH^{+}(aq) + H_2O_2(aq) \longrightarrow Cu^{I} + O_2^{-} + H^{+} \quad (5)$$

$$Cu^{II} + O_2^{-} \longrightarrow Cu^{I} + O_2 \quad (6)$$

The overall reaction (7) can be followed spectrophotometrically at 390 nm, the λ_{max} of $Cu(DMP)_2^{+}$.

$$4\,DMP + 2\,Cu^{II} + H_2O_2 \longrightarrow 2\,Cu(DMP)_2^{+} + O_2 + 2\,H^{+} \quad (7)$$

This method was used in our experiments to test the rate of H_2O_2 decomposition catalyzed by copper containing catalysts.

1.7. Research strategies

Based on previous reports of biomimetic binuclear copper centers, a new heterogeneous catalyst was chosen for investigation. While sol-gel methods can be used to construct silica materials with metal complex dopants, the small pores of these materials can restrict access to dopants for catalysis. In contrast, the surface-

immobilization method virtually guarantees that immobilized metal complexes are in accessible regions of the silica gel surface. Covalent attachment of dopants to the silica matrix (tethering) is normally achieved by adding molecules with pendant $-Si(OR)_3$ to silica. According to the method of Bowman, [21] silica gel can be modified with silylated ligands in the presence of copper.

Bis[(2-pyridyl)methyl]amine (BPA) was selected as the copper ligand for several reasons. First, BPA is a good ligand for copper ions because it has two pyridyl nitrogen atoms and one aliphatic amino nitrogen atom and thus a high affinity for copper ions. Importantly, however, it does not completely block the coordination sphere of the metal for interacting with additional substrate. Secondly, BPA is commercially available and readily modified reducing the synthetic burden. In addition, BPA is similar in structure to organic ligands that have shown oxidation chemistry in vitro when bound to copper.

Under basic conditions, binuclear copper centers are expected to form and serve as templates for ligand attachment to the silica surface. The active form of the catalyst is believed to be the μ-OH form (Figure 1.5).[21] As an initial gauge of the biomimetic potential of this new catalyst, catechol oxidase and catalase activities were investigated. Preliminary results indicate $Cu{\cdot}BPAS_4$ modified catalyst promotes both the biomimetic activities and warrants further study.

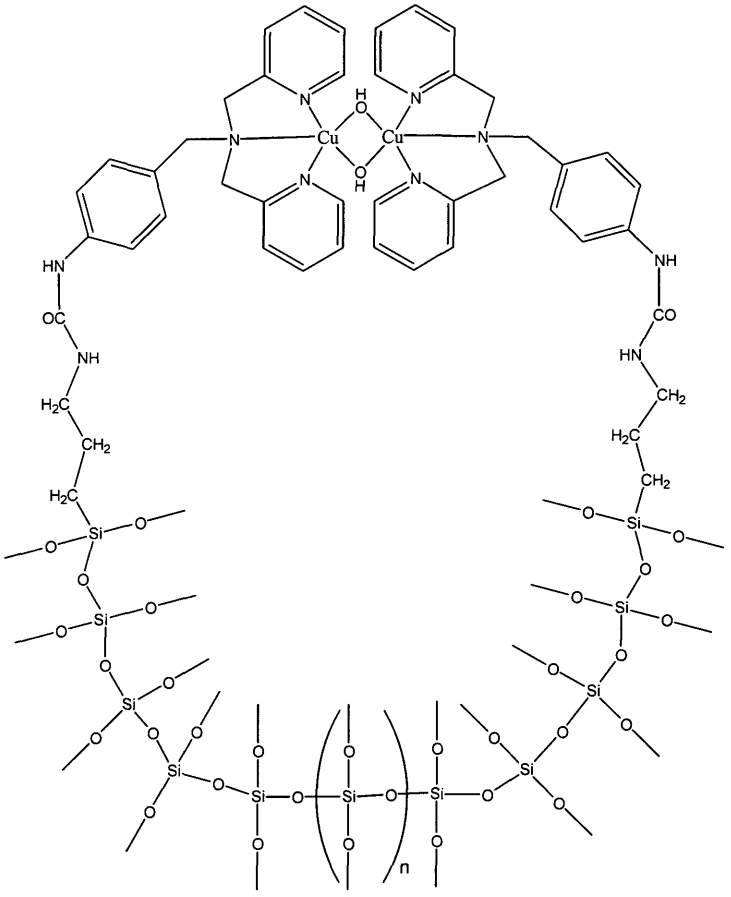


Figure 1.5: Expected structure of the catalyst in alkaline solution.

CHAPTER 2. EXPERIMENTAL

2.1. Instrumentation

A Perkin Elmer Lambda 35 UV/VIS Spectrometer was used to monitor copper catalysis of both DTBC oxidation and H_2O_2 decomposition. A Varian Mercury VX 400 MHz NMR was used to characterize organic materials.

2.2. Synthesis of 4-nitrobenzyl-bis[(2-pyridyl)methyl]amine ($BPAN_4$)

$BPAN_4$ was prepared by variation of the procedure described by da Mota and coworkers.[47] Bis[(2-pyridyl)methyl]amine (BPA) (4.98 mL, 27.75 mmol) was added with stirring to a suspension of sodium bicarbonate (2.33 g, 27.75 mmol) in 25 mL absolute ethanol. 4-Nitrobenzyl chloride (4-NBC) (5 g, 29.14 mmol) was added to the solution. The reaction mixture was refluxed under Ar for 40 hours. The precipitated sodium chloride was removed by vacuum filtration. The residue was acidified with 200 mL hydrochloric acid (1 M) and extracted with ether (2 x 100 mL). The aqueous phase was made basic with 20 mL NaOH (10 M) and then extracted with chloroform (5 x 100 mL). The combined organics were filtered, rotovapped, and dried under vacuum. The crude black solid was purified by liquid column chromatography (27 cm x 30 mm alumina, ethyl acetate: hexane = 1:1). $BPAN_4$ was isolated as a pale yellow solid (R_f = 0.18, mp 95 - 96 °C). Yield 94%. ^{1}H NMR ($CDCl_3$): δ 8.55 (d, 2 H, J = 5, H_a), 8.17 (d, 2 H, J = 9, H_h), 7.68 (t, 2 H, J = 8, H_c), 7.59 (d, 1 H, J = 9, H_g), 7.52 (d, 2 H, J = 8, H_d), 7.18 (t, 2 H, J = 6, H_b), 3.83 (s, 4 H, H_e), 3.81 (s, 2 H, H_f). The NMR spectrum is shown in Appendix I.

2.3. Synthesis of 4-aminylbenzyl-bis[(2-pyridyl)methyl]amine ($BPAA_4$)

$BPAN_4$ was reduced by $(NH_4)_2S$ to $BPAA_4$ according to the method of Bowman.[21] $(NH_4)_2S$ (20%, 70 mL) was added with stirring to a suspension of $BPAN_4$ (1.36 g, 4.07 mmol) in 87 mL ethanol (99%). The reaction mixture was refluxed under Ar for 9 hours at 60 °C. The ethanol was removed by rotary evaporation. The residue was acidified with 80 mL hydrocholoric acid (1M) and vacuum filtered through celite. The filtrate was extracted with ether (5 x 120 mL). The aqueous phase was made basic with 20 mL alkali (10 M) and extracted with chloroform (5 x 150 mL). The combined organics were rotovapped and dried under vacuum. The crude yellow solid was purified by recrystallization from toluene. Recrystallized $BPAA_4$ was a pale yellow crystal (mp 135 – 136 °C). Yield 67%. Elemental analysis for $C_{19}H_{20}N_4$, calculated: C 74.96%, H 6.63%, N 18.41%, observed: C 74.74 %, H 6.54%, N 18.32%. ^1H NMR ($CDCl_3$): δ 8.51 (d, 2 H, J = 5, H_a), 7.65 (t, 2 H, J = 8, H_c), 7.59 (d, 2 H, J = 8, H_d), 7.18 (d, 1 H, J = 8.5, H_g), 7.13 (t, 2 H, J = 6, H_b), 6.65 (d, 2 H, J = 8, H_h), 3.78 (s, 4 H, H_e), 3.56 (s, 2 H, H_f). The NMR spectrum is shown in Appendix II.

2.4. Synthesis of 4-nitrobenzyl-bis[(2-pyridyl)methyl]silylated amine ($BPAS_4$)

$BPAS_4$ was prepared by the addition of 3-(triethoxysilyl)propyl isocyanate (TSPI) to $BPAA_4$.[21] $BPAA_4$ (100 mg, 0.328 mmol) and TSPI (100 µL, 0.404 mmol, 1.23 equivalents) were dissolved in 20 mL $CHCl_3$. The reaction flask was immersed in a silicon oil bath and the pale yellow solution was stirred magnetically for 10 hours at 70 °C, replenishing the chloroform as it evaporated. Ten hours later, the oil bath temperature was raised to 80°C - 85°C for 1 hour to promote chloroform evaporation. The resulting residue (color ranged from yellow-orange to pale green for different batches) was dried in

vacuum for 2 days and used without further purification (mp 95 – 102°C). Yield 95%. Elemental analysis for $C_{29}H_{41}N_5O_4Si$, calculated: C 63.13%, H 7.49%, N 12.69%, O 11.60%, Si 5.09%, observed: C 61.31%, H 7.40%, N 12.69%. ^{1}H NMR ($CDCl_3$): δ 8.52 (d, 2 H, J = 5, H_a), 7.67 (t, 2 H, J = 8, H_c), 7.57 (d, 2 H, J = 8, H_d), 7.34 (d, 1 H, J = 8, H_g), 7.23 (d, 2 H, J = 8.5, H_h), 7.15 (t, 2 H, J = 6, H_b), 3.80 (q, 4 H, J = 7, H_l), 3.79 (s, 2 H, H_e), 3.63 (s, 2 H, H_f), 3.24 (q, 2 H, J = 6, H_i), 1.63 (q, 2 H, J = 8, H_j), 1.21 (t, 2 H, J = 7, H_m), 0.63 (t, 2 H, J = 8, H_k). The NMR spectrum is shown in Appendix III.

2.5. Treatment of silica gel with HCl/HNO_3

Trace impurities present in commercial silica gel were removed by stirring for one hour in a 6 M HCl/ 6 M HNO_3 solution.[49] The acid treated silica gel was collected by vacuum filtration and rinsed with deionized water until the suspension was pH 7.0, and dried in the oven at 110 °C overnight.

2.6. Preparation of modified silicas at different pH values

2.6.1. Modification of silica by $BPAS_4$ prior to Cu(II) addition

$BPAS_4$ in methanol (1 mL, 0.02 M), and deionized water (2 mL) were added to 1 g acid treated silica. The pH of the mixture was adjusted to 8, 9, 11, 12, or 13 with 1 M NaOH. The suspension was stirred for 1 hour before rinsing with 500 mL H_2O: Methanol (2:1) to obtain a neutral filtrate. The modified silica was dried in the air overnight. The dried silica was collected, weighed, mixed with a stoichiometric amount of aqueous copper nitrate (100% $BPAS_4$ incorporation assumed), rinsed with 500 μL deionized H_2O, and dried in the air overnight.

2.6.2. Modification of silica by $BPAS_4$ in the presence of Cu(II)

$BPAS_4$ in methanol (1 mL, 0.02 M), and an aqueous solution of $Cu(NO_3)_2$ (2 mL, 0.01 M) were added to 1 g acid treated silica. The pH of the mixture was adjusted to 8, 9, 10, 11, 12, or 13 with 1 M NaOH. Modified silica was rinsed with 500 mL H_2O : methanol (2:1) to obtain a neutral filtrate, and dried in the air overnight.

2.7. HCl assay for [Cu]

2.7.1. Calibration curve for [Cu] in concentrated HCl

Stock solutions of $Cu(NO_3)_2$ (0.04 mM, 0.08 mM, 0.10 mM, 0.12 mM, 0.16 mM, 0.20 mM) in concentrated HCl (12.1 M) were made from standard $Cu(NO_3)_2$ (15.7 mM in 2% HNO_3). Absorbance was measured at 384 nm. [49, 50] Deionized water was used as the blank.

2.7.2. [Cu] in acid treated and modified silica

Concentrated HCl (5 mL, 12.1 M) was added to around 30 mg of acid treated or modified silica, and the mixture was shaken and set aside for one hour to ensure the complete removal of Cu(II) from the silica surface. After the silica settled out, the acidic supernatant was pipetted off with glass pipettes for the measurement of absorbance at 384 nm.[48] Deionized water was used as the blank. Generally, dilution with 5 or 10 mL HCl was required to achieve an absorbance reading between 0.1 and 0.5 ($\varepsilon_{384nm} = 2169\ M^{-1}cm^{-1}$).[49, 50]

2.8. 2,9-Dimethyl-1,10-phenanthroline assay for [Cu] and [H_2O_2]

2.8.1. [Cu]

The concentration of aqueous copper nitrate were determined by adding sufficient aliquots of the stock solution to excess 2,9-dimethyl-1,10-phenanthroline

(DMP) and ascorbate.[18, 50, 51] Formation of [Cu(2,9-*di*-methyl-1,10-phenanthroline)$_2$]$^{1+}$ was monitored at 454 nm, and concentration were determined based on $\varepsilon_{454nm} = 7950$ $M^{-1}cm^{-1}$.[47]

2.8.2. [H_2O_2]

The concentration of H_2O_2 was determined based on its ability to reduce an excess amount of [Cu(2,9-di-methyl-1,10-phenanthroline)$_2$]$^{2+}$.[18, 46] First, H_2O_2 (300 μL, 30%) was added to the mixture of sufficient catalyst (around 300 mg) to provide 3.93 μmol Cu(II) and 5700 μL deionized H_2O. The reaction mixture was continuously stirred magnetically in air at room temperature. Every ten minutes during the first two hours of the reaction, 50 μL aliquots were removed, diluted in 950 μL H_2O and centrifuged in eppendorf tubes, from which 200 μL liquid mixture was transferred to 800 μL H_2O and mixed to achieve a 100 fold dilution. A 20 μL portion of the diluted solution was transferred to a vial containing 300 μL aqueous $Cu(NO_3)_2$ (0.026 M), 1610 μL methanolic DMP (0.01 M) and 1070 μL deionized H_2O. Additional aliquots were assayed at 3, 4, 5 hours elapsed time. Complete decomposition of H_2O_2 always occurred in less than 24 hours in the presence of copper containing modified silica.

2.9. 3,5-di-*tert*-butylcatechol (DTBC) oxidation

Catechol oxidase activity was determined by the oxidation rate of DTBC at room temperature. DTBC (10 mL, 20 mM in methanol) was added to sufficient catalyst (around 30 mg) containing 0.39 μmol Cu(II). The reaction mixture was continuously stirred in air at room temperature. Every two minutes during the first twenty minutes of the reaction, 1 mL upper liquid was removed and measured at 390 nm (ε = 1900 $M^{-1}cm^{-1}$), λ_{max} for 3,5-di-*tert*-butylquinone (DTBQ), the oxidation product.[51]

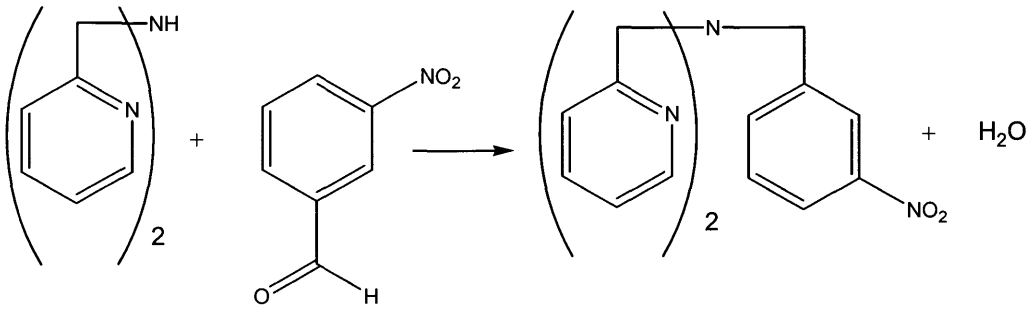
CHAPTER 3. RESULTS AND DISCUSSION

3.1. Tethered ligand synthesis

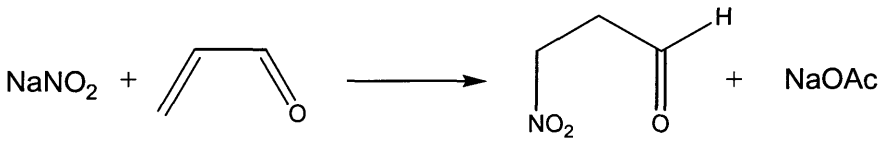
Covalent incorporation into a silica matrix is most readily accomplished by displacement of alkoxy group from alkoxysilane-functionalized ligands by the hydroxyl group of silica. Commercially available 3-(triethoxysilyl)propyl isocyanate (TSPI) is suitable for development of tethered ligands when an auxiliary amine group is present to form a urea linkage.[21] Since direct reaction of TSPI with the aliphatic amine nitrogen of BPA would greatly diminish its metal affinity, alkylation of the secondary amine with a compound containing a remote linkage group was desired.

Previous work in the Bebout laboratory had evaluated three synthetic strategies for the derivatization of BPA. Originally, auxiliary amine introduction through a one step process involving addition of aziridine to BPA was contemplated, but aziridine is prohibitively expensive and unstable. Since direct alkylation of BPA with a bifunctional amine is not possible, a two step addition and nitro group reduction strategy was adopted. The first attempt to use this strategy involved coupling *para*-nitrobenzaldehyde to BPA.[52-54] Unfortunately, the conversion of the secondary amine to the tertiary amine was low. Variations of experimental parameters, including excess mole equivalents of nitrobenzaldehyde, extended reactions time, higher reaction temperature, different solvents and conducting the reaction in the presence of anhydrous magnesium sulfate, did not enhance conversion. Since deactivation of the aldehyde functionally by the *para*-nitro

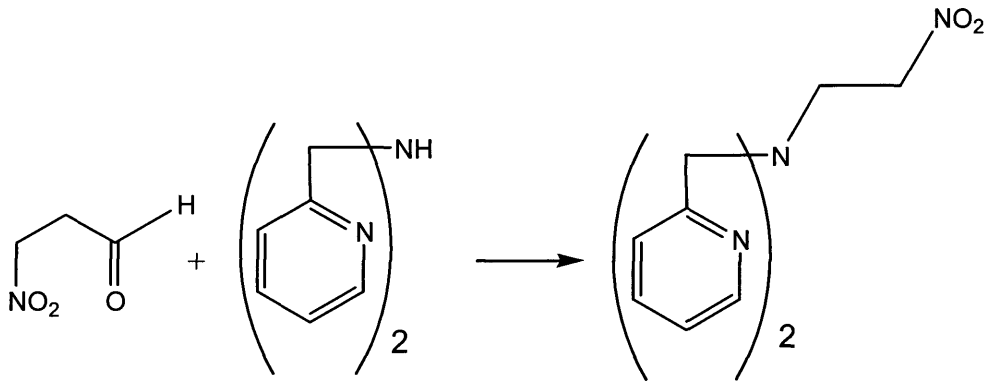
group was conceivable, the related coupling with *meta*-nitrobenzaldehyde shown below was also investigated, but conversion remained low.



Suspecting the aldehyde was deactivated by conjugation, the next strategy tried involved reaction of acrolein with sodium nitrate to produce the saturated aldehyde 3-nitropropanal:[55]

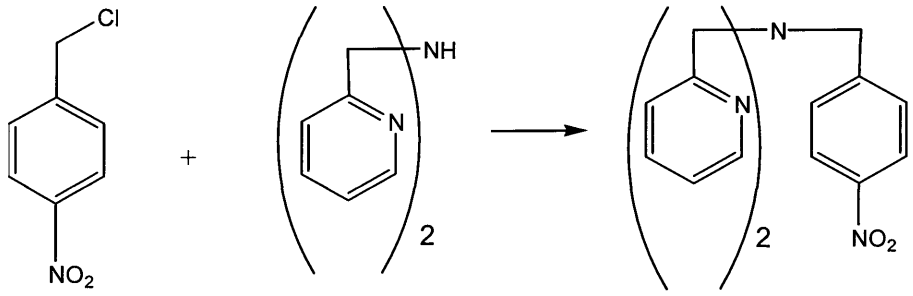


Reaction of BPA with 3-nitropropal was expected to provide a tertiary amine with tethered nitro functionality:[56]



However, this reaction produced a complex mixture of products. Furthermore, the purification of 3-nitropropanal was complicated and the yield was low, so an alternative procedure was investigated. The commercially available bifunctional compound 4-

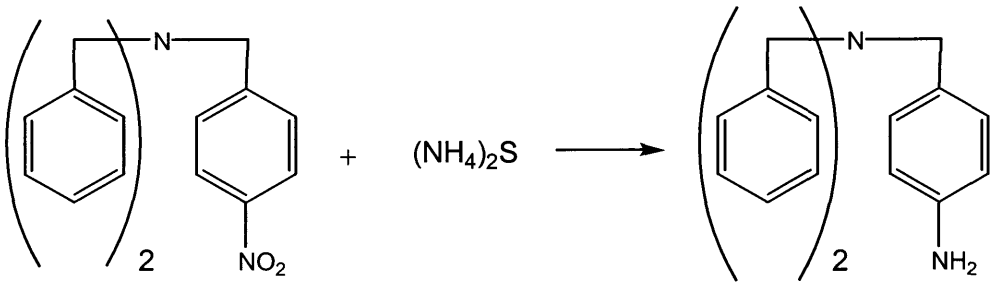
nitrobenzyl chloride (4-NBC) was expected to undergo S_N2 attack by the aliphatic amine nitrogen of BPA to give $BPAN_4$:[47]



4-NBC BPA $BPAN_4$

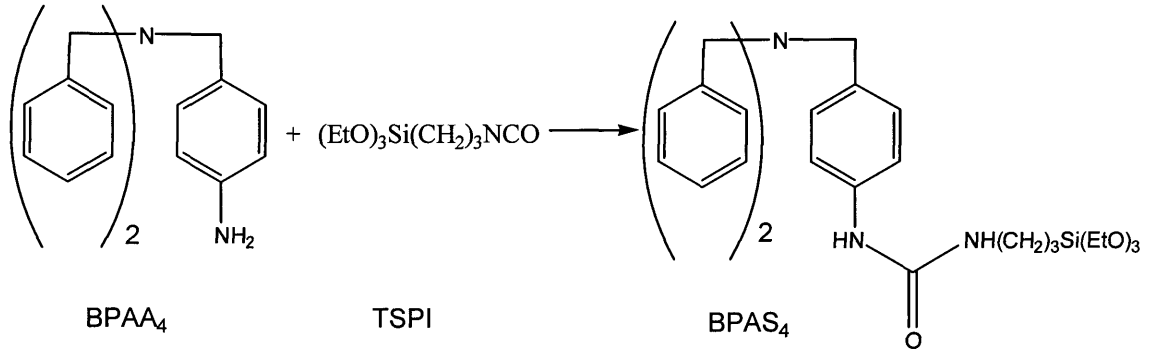
Under optimized reaction conditions involving 40 hours of reflux in ethanol in the presence of $NaHCO_3$, high conversion to $BPAN_4$ was observed. Alumina chromatography with 50% ethyl acetate/ 50% hexanes removed highly colored byproducts from the $BPAN_4$ (90% yield).

To complete the synthesis of the ligand tether, reduction of the $BPAN_4$ nitro group to a primary amine ($BPAA_4$) was first effected with ammonium sulfide in ethanol heated to 60 °C for 9 hours:



$BPAN_4$ $BPAA_4$

Nucleophilic addition of the primary amine of $BPAA_4$ to the isocyanate group of TSPI by the method of Bowman[21] formed a urea linkage, producing the silylated urea $BPAS_4$ (95% yield). Elemental analysis indicated the products $BPAA_4$ and $BPAS_4$ were quite pure.



Due to the sensitivity of silylethers to hydrolysis, the workup of $BPAS_4$ was limited to solvent removal. Although essentially identical procedures were associated with each $BPAS_4$ synthesis, the color of $BPAS_4$ ranged from yellow to orange to pale green.

3.2. Preparation of modified silicas

As discussed in the introduction, the surface-immobilization method was used to prepare the catalyst. Since the potential of using a base promoted binuclear copper center as a template for ligand tethering is our focus, two variations in the tethering procedure were investigated:[20, 39]

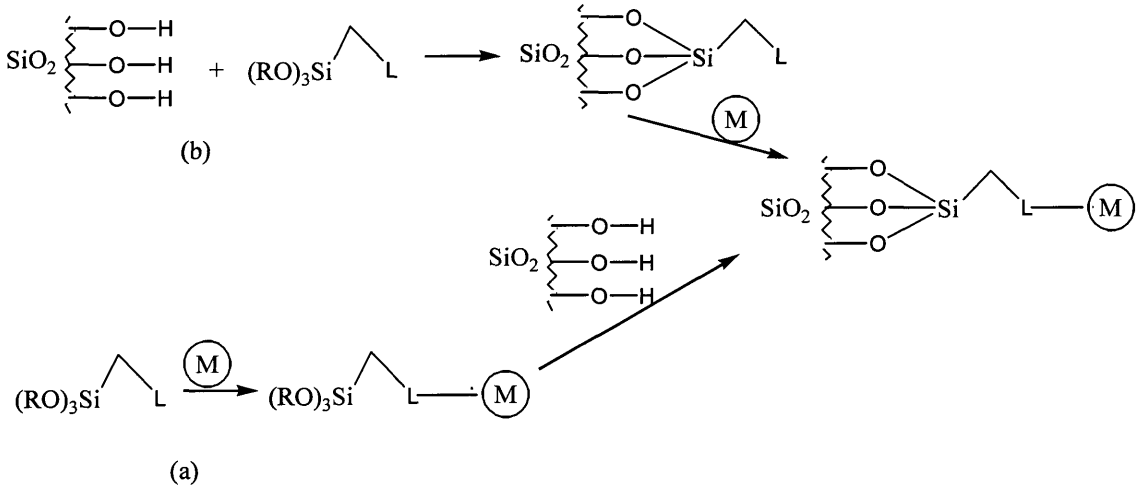


Figure 3.1: Two routes used for preparing silica-immobilized transition metal complexes. (Ref: 20, 39)

In method (a), the alkoxysilane-functionalized ligand is first used to functionalize a silica surface. The modified material is then mixed with a metal salt to generate a supported metal complex. In method (b), the silylated metal complex is stirred with the support material to allow for covalent attachment to the silica. The later conditions have the potential to alter the ligand distribution on the surface and change the properties of the materials. The copper contents, catechol oxidase and catalase activities of silica prepared by both methods described above were characterized.

The pH dependence of the catalytic activity of the modified silica was investigated over the range of $8 < pH < 13$. Modified silicas were rinsed with deionized water until filtrates became neutral to minimize possible pH effects on catalysis. Adjustment of pH with 10 M NaOH produced materials with catalase activity initial lag periods in excess of twelve hours. These undesirable lag periods in catalase activity were not observed when 1 M NaOH was used for pH adjustment. In addition, silica recovery was typically 95% when the modification pH was less than 12 and decreased to 20% at pH 13. As a result, studies conducted with silica modified at pH 13 were limited.

3.3. [Cu] in silica materials

Taylor[50] determined that the easiest method for determination of the copper content of silica materials involved treatment with concentrated HCl. This treatment protonates the copper ligands and produces $CuCl_6^{4-}$, which absorbs at 384 nm in concentrated HCl. The molar absorptivity reported in the literature for Cu(II) by this method was 1544 $M^{-1} \cdot cm^{-1}$. A standard curve for the determination of [Cu] by this method was generated using a standard $Cu(NO_3)_2$ solution (15.7 mM in 2% HNO_3).

Serial dilution of the standard reproducibly generated a calibration curve indicating a molar absorptivity of 2169 $M^{-1} \cdot cm^{-1}$ (Figure 3.2).

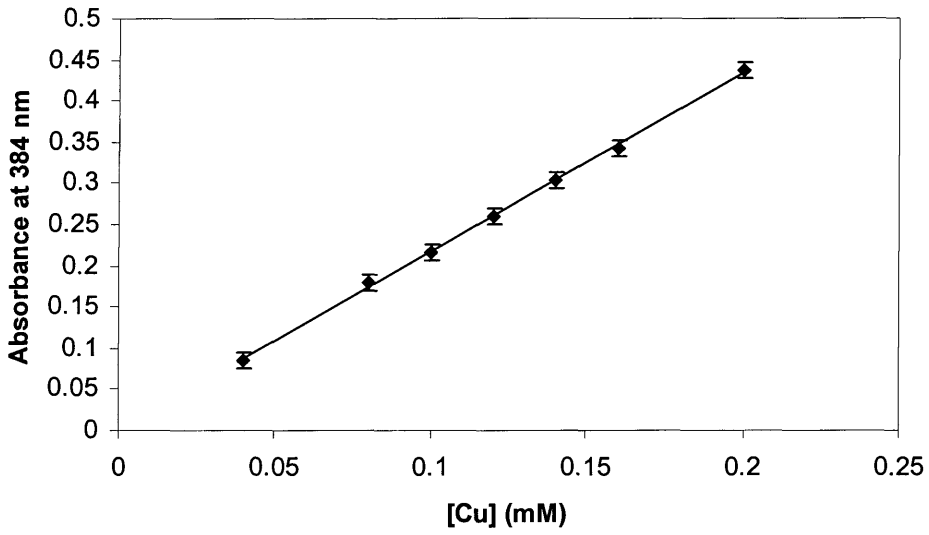


Figure 3.2.Calibration curve of copper nitrate by 12.1 M HCl analysis.

Since the smaller literature molar absorption led to unreasonable copper contents (greater than the total amount of copper added to the silica), the empirical value was used. The reasons for the disparity between the experimental and literature molar absorptivity were not investigated. The procedure for determination of the copper content of silica materials involved suspending approximately 30 mg in 5 mL concentrated HCl with measures to minimize evaporation. Based on Beer's law, copper content in terms of μmol Cu/ mg silica can be calculated with the expression:

$$[Cu] = \frac{A_{384nm} \cdot 5\ mL}{2.169\ mM^{-1}cm^{-1} \cdot 1\ cm \cdot mg\ silica}$$

The copper content of acid treated silica was determined to be 0.2 nmol Cu/mg. Instability of absorbance readings for concentrated HCl solutions place error bars of ±0.1

nmol Cu/mg on this value. As a control experiment, the copper contents of $BPAS_4$ modified silicas were examined. According to the concentrated HCl assay, there were trace amounts of copper present in these materials but the levels were generally negligible compared to materials to which copper was added and only slightly higher than acid treated silica (Table 3.1). Differences in copper content of modified silicas prepared by the two methods were relatively small compared to differences of up to 30% in copper content of modified silica prepared from different batches of $BPAS_4$. These results indicate that neither pH nor [Cu] had an impact on silica modification.

Table 3.1. Representative [Cu] in $BPAS_4$ modified silica and catalysts prepared by method (a) and (b)

	[Cu] (±1.4 nmol/mg)				
	pH 8	pH 9	pH 10	pH 11	pH 12
$BPAS_4$ modified silica	3.2	0.6	0.5	1.1	0.5
Method (a) catalysts	11.8	12.8	12.9	14.0	13.1
Method (b) catalysts	12.5	12.1	X	14.7	12.9

3.4. Investigation of biomimetic catecholase activity

Based on the work of other researchers,[8-10, 37] the catechol oxidase activity of copper(II) complexes are dependent on the presence of hydroxide bridged binuclear copper centers with relatively short intra copper separation. Structures of this kind can be obtained in synthetic system by pH modification. Optimum catalytic activity for previously reported complexes occurred at pH 13 or 14.[21] Similarly, the catalyst synthesized in our experiments was anticipated to have higher catechol oxidase activity

with increasing pH. The oxidation rate of DTBC was directly obtained by monitoring the absorbance at 390 nm (ε = 1900 $M^{-1}cm^{-1}$), the λ_{max} for DTBQ.[51]

The time dependent absorbance data for DTBC oxidation at room temperature for separate batches of Cu·$BPAS_4$ modified silica are shown in Figure 3.3. Similarities in catalytic behavior between modified silicas made with different batches of $BPAS_4$ were limited. Batches of catalyst made from the same batch of $BPAS_4$ provided reproducible results. It may be relevant that batches of $BPAS_4$ varied in color, perhaps due to small amount of metal contamination. Factors leading to different batch to batch activities will be the focus of future studies.

For silicas modified with Cu·$BPAS_4$ at pH 8 and 9, we consistently observed a linear correlation between time and DTBC oxidation. In contrast, silica modified at higher pH exhibited both linear and non-linear time dependent DTBC oxidation. Significantly, the modified silicas prepared at pH 8 and pH 9 had very low catalytic activity. The modified silicas prepared at higher pH generally had higher catalytic activity. Unfortunately, there was a higher degree of variability in the catalytic activity of silicas modified at high pH.

Cu(II) loading on silica was evaluated as a potential factor affecting the catechol oxidase activity. Rate of change in absorbance at 390 nm with time for DTBC oxidation from the four batches of catalyst shown in Figure 3.3 were determined from the linear or pseudo-linear part of data to be reproducible and plotted against the Cu(II) loading on silica (Figure 3.4.). No trend between [Cu] and slope was evident. Future studies will address the reproducibility of silica immobilization and other parameters, which might alter DTBC oxidation behavior.

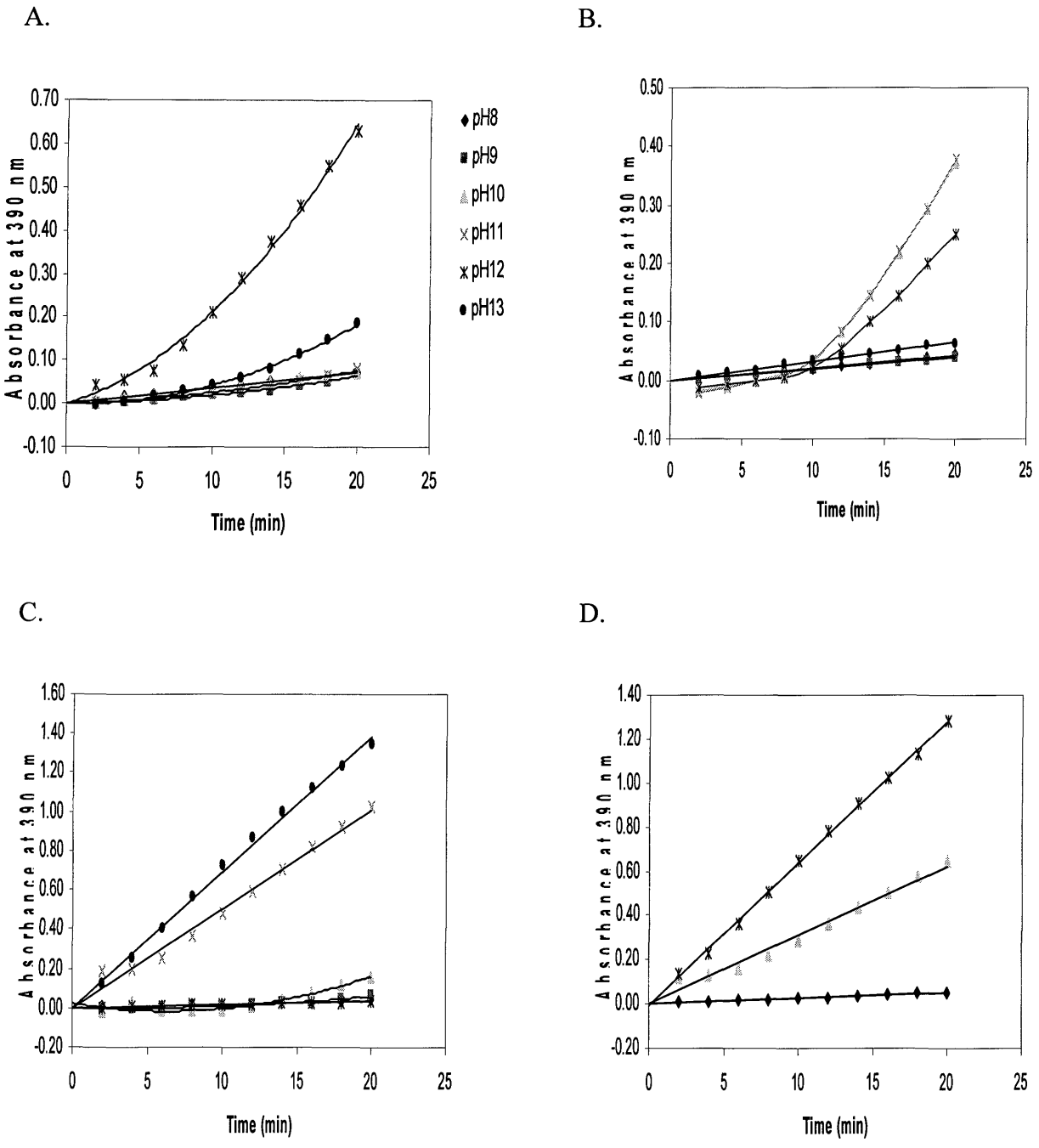


Figure 3.3: Absorbance at 390 nm vs time for DTBC oxidation in the presence of Cu·$BPAS_4$ modified silica. Catalysts were prepared by method (a) at the pH indicated. Each plot corresponds to a set of catalysts prepared from one batch of $BPAS_4$. DTBC (10 mL, 20 mM) was incubated with $BPAS_4$ and Cu(II) modified silica containing 0.39 μmol Cu(II) at room temperature.

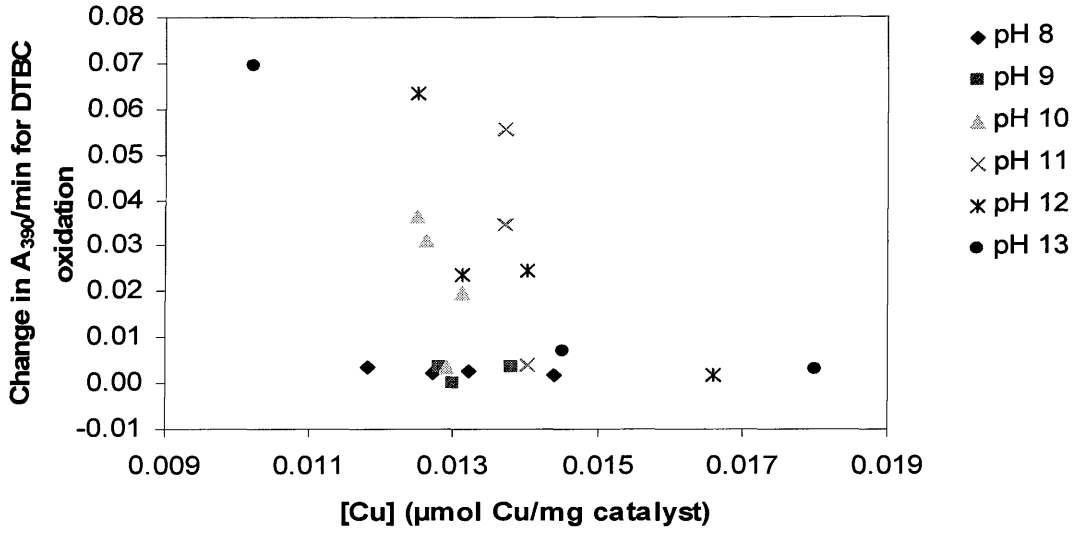


Figure 3.4: Rate of change in absorbance at 390 nm with time for DTBC oxidation vs Cu(II) loading on silica at different pH. The rate of change of absorbance was determined from the linear or pseudo-linear part of data. DTBC (10 mL, 20 mM) was incubated with $BPAS_4$ and Cu(II) modified silica containing 0.39 µmol Cu(II) at room temperature.

Averaging the rates of change in absorbance at 390 nm with time for DTBC oxidation from these four different batches suggested a possible trend. The catecholase activity improved with increasing pH of silica modification until pH 11 the decreased. While pH 11 may be the ideal silica modification pH for optimum catacholase activity, the standard deviations in the pH 10 to 13 data indicate considerable variations between batches (Figure 3.5). Future studies will examine the catalytic potential of several batches of Cu·$BPAS_4$ modified silica prepared from the same $BPAS_4$ prep.

3.5. Investigation of biomimetic catalase activity

Generally, copper(II) amine and imine complexes form hydroxo-bridge dinuclear structures at high pH.[57-60] According to the mechanism proposed by Luo in 1988,[45] OH^- plays an important role in H_2O_2 decomposition catalyzed by copper complexes. To

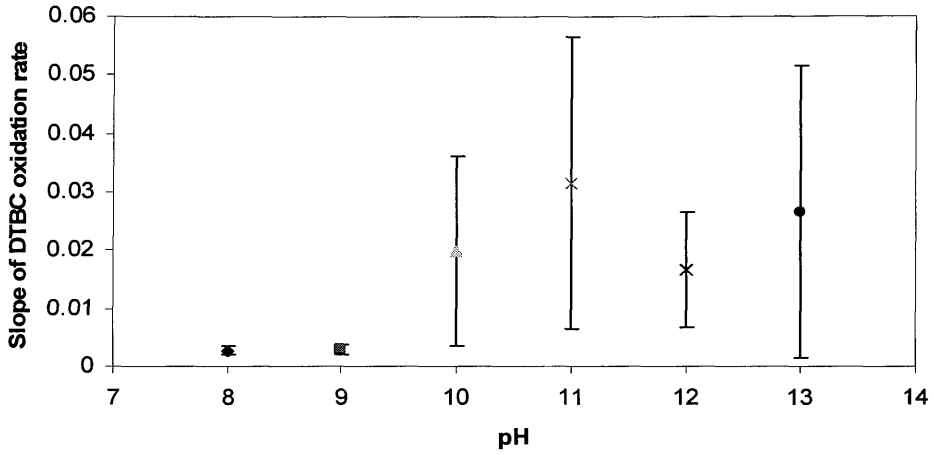


Figure 3.5: Average DTBC oxidation rate vs pH. The standard deviations in the observed slopes are indicated by the error bars.

examine the role of hydroxylated forms of copper-$BPAS_4$ in H_2O_2 decomposition, the homogeneous complex at pH 8 to 13, as well as silicas modified by methods (a) and (b) over this pH range (Figure 3.1) were evaluated for their catalytic potential. The H_2O_2 decomposition rate was indirectly monitored by the reduction of Cu^{II}-DMP complexes (DMP is the bidentate ligand 2, 9-dimethyl-1, 10-phenanthroline). The formation of $Cu(DMP)_2^+$ was monitored at 454 nm.

Catalysts prepared by both methods promoted H_2O_2 decomposition above the background rate of spontaneous H_2O_2 degradation, which is slow at room temperature. The decomposition of H_2O_2 was typically complete in less than 24 hours in the presence of modified silica prepared by either method (a) or (b). However, when modified silicas were prepared by method (a) without pH adjustment, H_2O_2 decomposition over 24 hours was reduced to less than 70 %. The reasons for this behavior will be the focus of a future study. The rate of decomposition was approximately linear during the first 60 minutes (< 10% overall H_2O_2 consumption) (Figure 3.6). For silica modified by Method (a), preparation at higher pH generally produced more active H_2O_2 decomposition catalysts

(Figure 3.6) with a maximum at pH 12. There was very little difference in the catalytic activity of silica prepared at varying pH by method (b) (Figure 3.7). The catalytic activity of modified silica prepared by method (a) was consistently higher than materials prepared by method (b), consistent with the proposed role for copper templating on catalyst activity. As an additional control experiment, the catalase activity of unsupported Cu(II)·BPA was examined at pH 10. In Figure 3.8, the behavior of the heterogeneous catalyst prepared at pH 10 and homogeneous system at pH 10 during the first 60 minutes is compared. The rate of H_2O_2 decomposition was 2.2 times faster with the heterogeneous catalysts. This suggests that silica immobilization enhances the stability of the active catalysts, but additional trials are needed to confirm the generality of this behavior.

A: B:

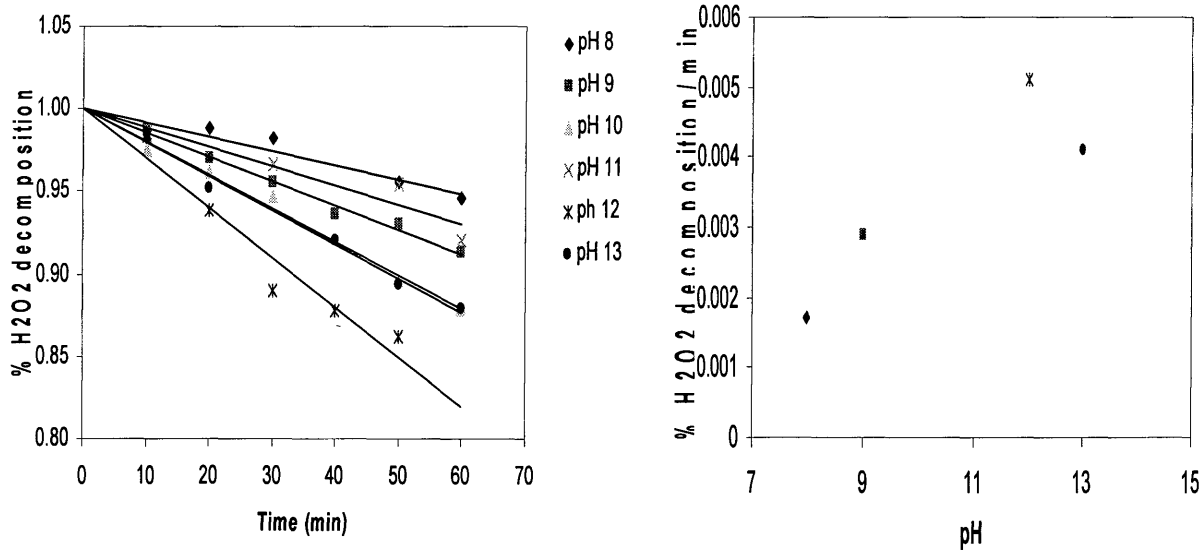


Figure 3.6: H_2O_2 decomposition catalyzed by Cu(II)-$BPAS_4$ modified catalyst prepared by method (a) at the pH indicated. Decompositions were conducted as described in section 2.8.3. A: % decomposition of H_2O_2 as a function of time. B: % decomposition of H_2O_2/min as a function of the pH at which silica was modified by Cu(II)-$BPAS_4$.

A: B:

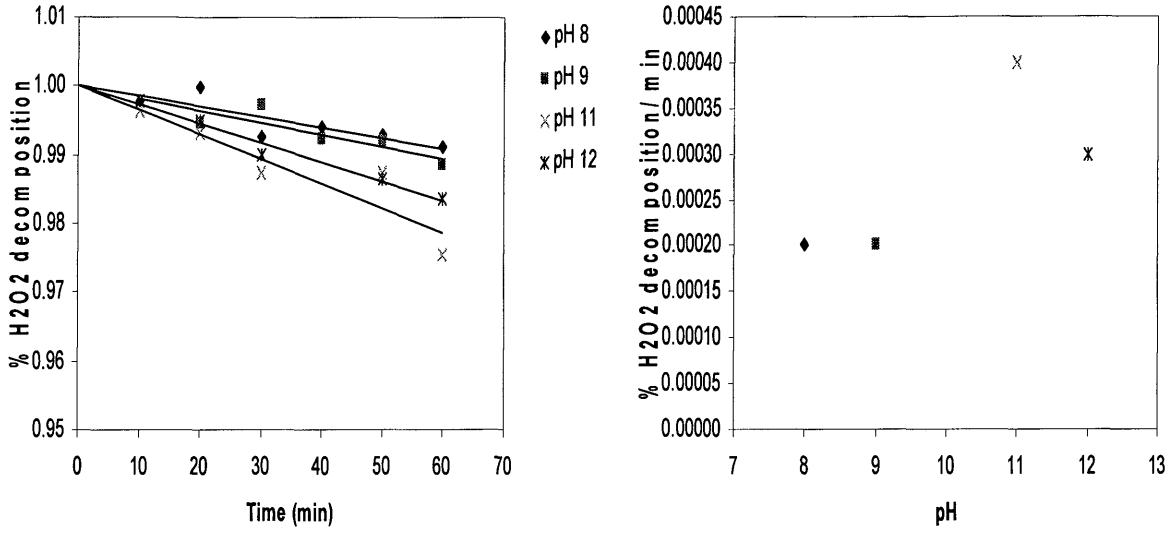


Figure 3.7: H_2O_2 decomposition catalyzed by Cu(II)-$BPAS_4$ modified catalyst prepared by method (b) at the pH indicated. Decompositions were conducted as described in section 2.8.3. A: % decomposition of H_2O_2 as a function of time. B: % decomposition of H_2O_2/min as a function of the pH at which silica was modified by Cu(II)-$BPAS_4$.

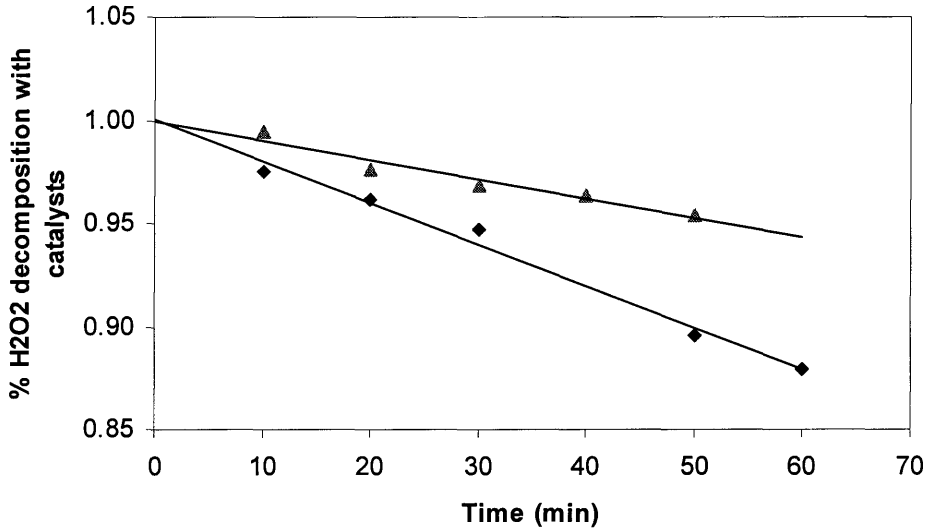


Figure 3.8: Comparison of catalase activity of Cu(II)-$BPAS_4$ modified catalyst prepared at pH 10 by method (a) ([Cu] = 2.6 mM) and a homogeneous solution of BPA and $Cu(NO_3)$ ([BPA] : $[Cu(NO_3)_2]$= 1:1, [Cu] = 2.6 mM). Decompositions were conducted as described in section 2.8.3. Somang Kim provided the homogeneous catalysis data. ◆catalyst prepared by method (a) ▲a homogeneous solution of BPA and $Cu(NO_3)_2$.

CHAPTER 4. CONCLUSION

The biological role of copper has been studied for long time. Many biomimetic copper complexes have been synthesized and their catechol oxidase and catalase activities investigated. In this project, a silylated tridentate amine was synthesized by derivitization of bis[(2-pyridyl)methyl]amine (BPA), for preparation of a heterogeneous copper catalyst. Since active forms of previous biomimetic copper complexes typically have μ-OH and basic pH is known to promote this feature, modified silicas were prepared over the pH range of 8 to 13. The effect of the pH of silica modification on catalysis of 3,5-di-*tert*-butylpyrocatechol (DTBC) oxidation (catechol oxidase activity) was examined. Two different methods to prepare the catalyst were also studied to investigate the potential of hydroxyl bridged binuclear copper centers to serve as templates during silica modification. In the first method, copper was present during silica modification with $BPAS_4$, while in the second one, the silica modified with $BPAS_4$ was mixed with a copper nitrate solution after pH adjusting. Silicas modified by both methods at various basic pH values were evaluated as catalyst of H_2O_2 decomposition (catalase activity).

Concentrated HCl treatment provided a convenient method to determine copper loading in silica. The molar absorption (2169 $M^{-1}\cdot cm^{-1}$) at 384 nm, the λ_{max} for $CuCl_6^{4-}$, was experimentally much higher than the literature value (1544 $M^{-1}\cdot cm^{-1}$). A standard curve was reproducibly made by using serial dilution of a standard $Cu(NO_3)_2$ (15.7 mM in 2% HNO_3) to determine [Cu]. Since the smaller literature molar absorption caused

unreasonable higher copper contents than the total amount of copper added to the silica, the empirical value was used. The reason for this discrepancy was not studied, but it could not be the evaporation of the standard $Cu(NO_3)_2$ solution since that would decrease the molar absorption value.

Determination of the copper content of silicas modified with $BPAS_4$ in the presence and absence of copper revealed differences of up to 30% over the pH range examined. Since there were significant differences between copper contents of catalyst batches prepared under similar conditions, catalyst amounts were based on providing a fixed amount of copper. However, there was no obvious trend in copper content for silicas modified with $BPAS_4$ at different pH or copper concentrations, suggesting neither of the parameters have an impact on silylation.

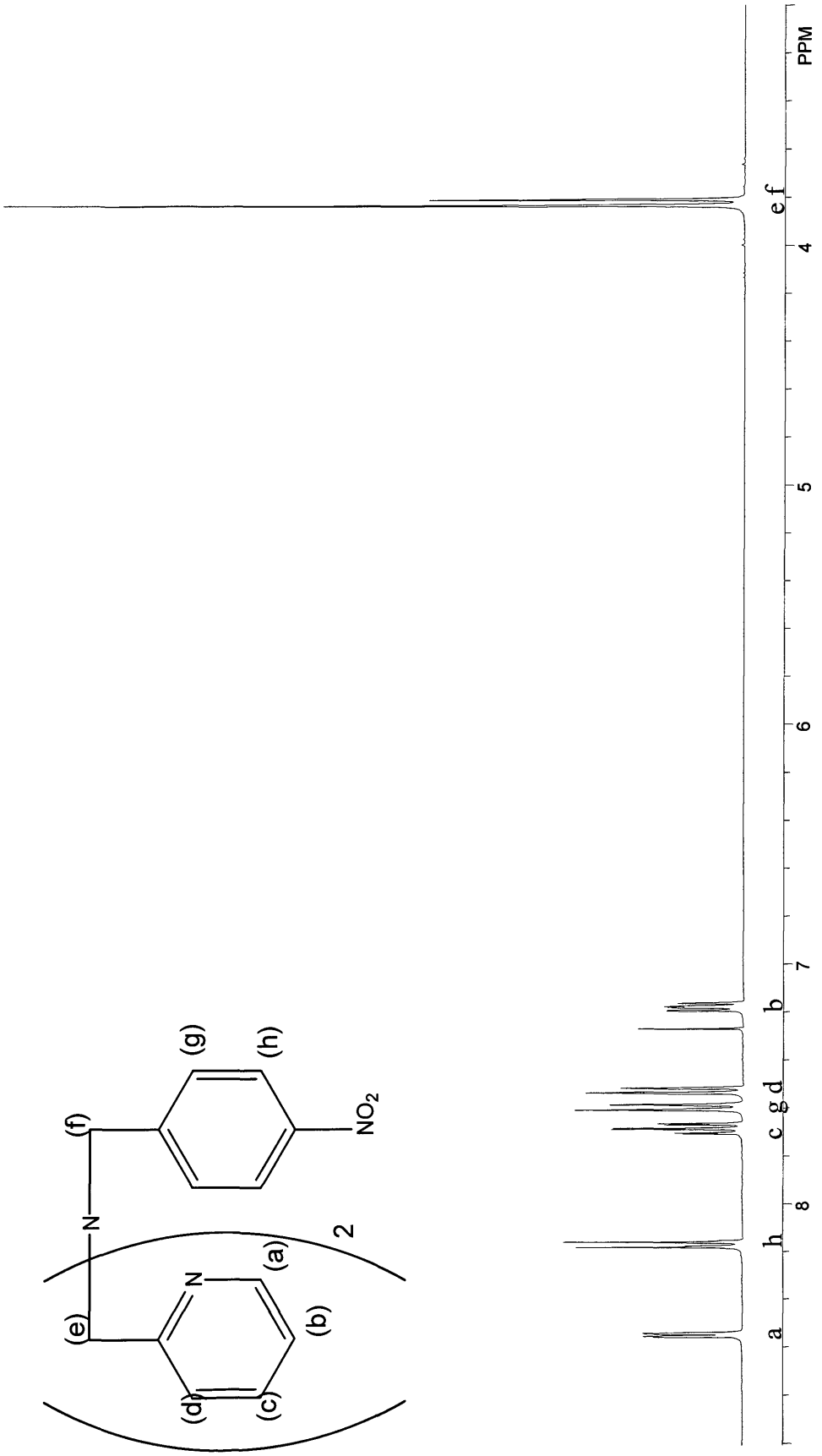
To investigate the catachol oxidase activity of $Cu{\cdot}BPAS_4$ modified silicas, 3, 5-di-*tert*-butylpyroquinone (DTBQ), produced by oxidation of DTBC, was monitored at 390 nm. There are at least two factors, pH and copper loading, which possibly affect the catechol oxidase activity. Based on literature present, the μ-OH form of the catalyst was believed to be active. Similarities in catalytic behavior between modified silicas made with different batches of $BPAS_4$ were limited, but batches of catalyst made from the same batch of $BPAS_4$ provide reproducible results. Silica modified of low pH (8 and 9) had low catalytic activity. Silica modification at pH 13 led to poor silica recovery, suggesting significant destruction of silica structure and complicating interpretation of the catalytic activity. Among pH 10, 11, and 12, inconsistents in activity between batches precluded determination of an optimum pH. Copper loading for catalyst modified at different pH was also studied, but no relationship was found between DTBC oxidation rate and [Cu]

in silica. In addition, different batches of $BPAS_4$ gave rise to different levels of catalytic activity. More experiments under different conditions for the same batch would possibly help identify relevant experimental parameters needing to be controlled to improve reproducibility. Development of procedures for preparing larger batches of both $BPAS_4$ and modified silica will facilitate these studies.

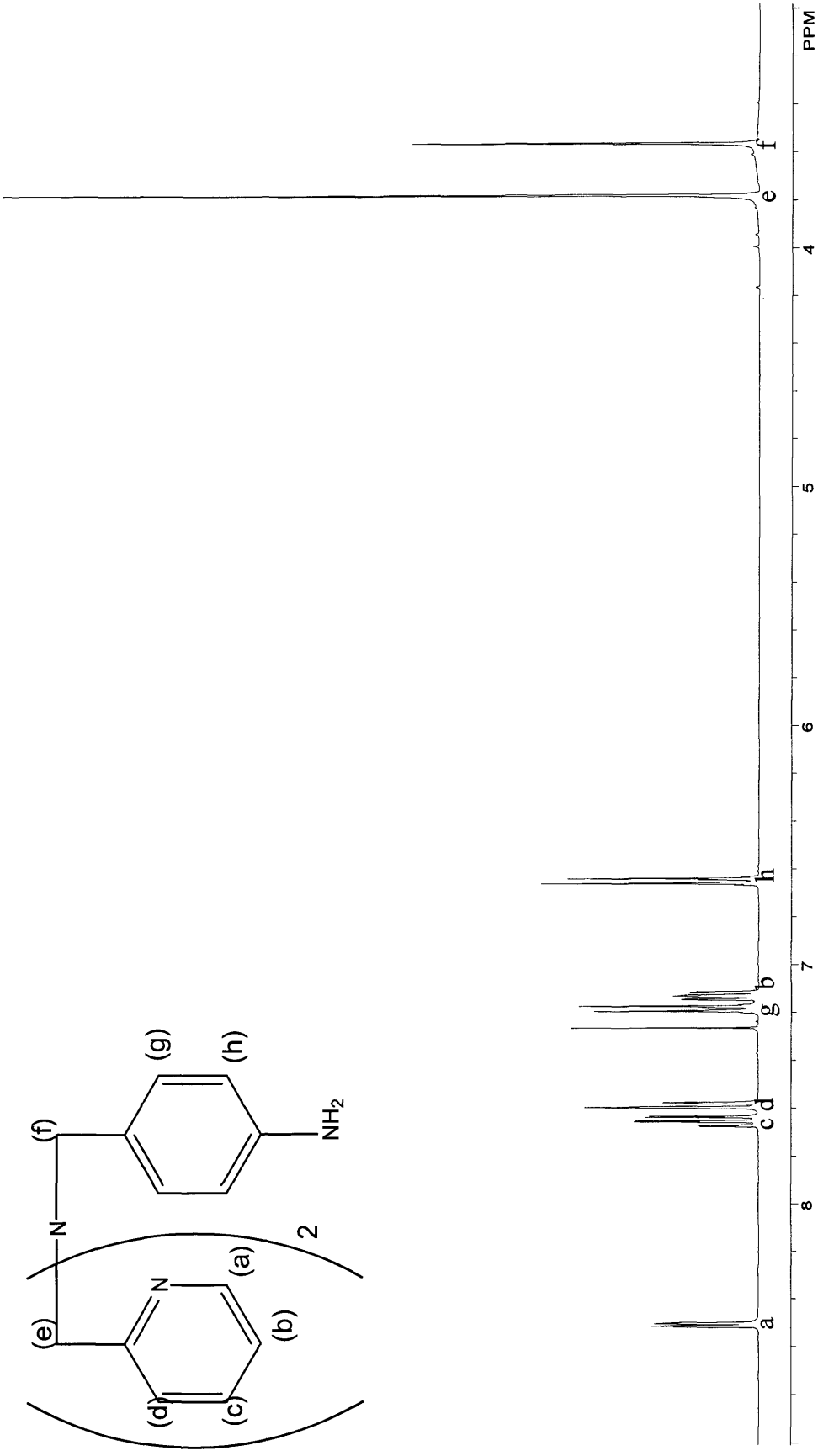
To investigate the catalase oxidase activity of $Cu{\cdot}BPAS_4$ modified silicas, $Cu(DMP)_2^+$, produced by reduction of $Cu(DMP)_2^{2+}$, was monitored at 454 nm. The decomposition rate of H_2O_2 was calculated from the rate of reduction of $Cu(DMP)_2^{2+}$ to $Cu(DMP)_2^+$. The effect of the pH used for silica modification was more obvious than for DTBC assay. If copper was present during $BPAS_4$ modification of silica (method (a)), catalyst effectiveness generally increased from 8 to 12. If copper was added following $BPAS_4$ modification of silica (method (b)), there was almost no difference in catalase activity for silicas modified at different pH. These results suggest that base promoted formation of μ-hydroxyl binuclear copper centers is critical for the production of the active form of the catalyst.

These studies suggest that $Cu{\cdot}BPAS_4$ modified silicas have potential as biomimetic catalysts, but more studies are clearly needed. Development of methods which produce catalyst with consistent properties is a top priority of future studies. Also, the reproducibility of results under specific conditions will be addressed. In addition to copper loading and the pH of silica modification, other factors will be tested for their contribution to the catalytic activities. Also, variations in the behavior of catalyst for DTBC oxidation, including reasons for early inactivity in some batches, will be assessed. Future studies may also attempt to determine the structures of the copper complexes with

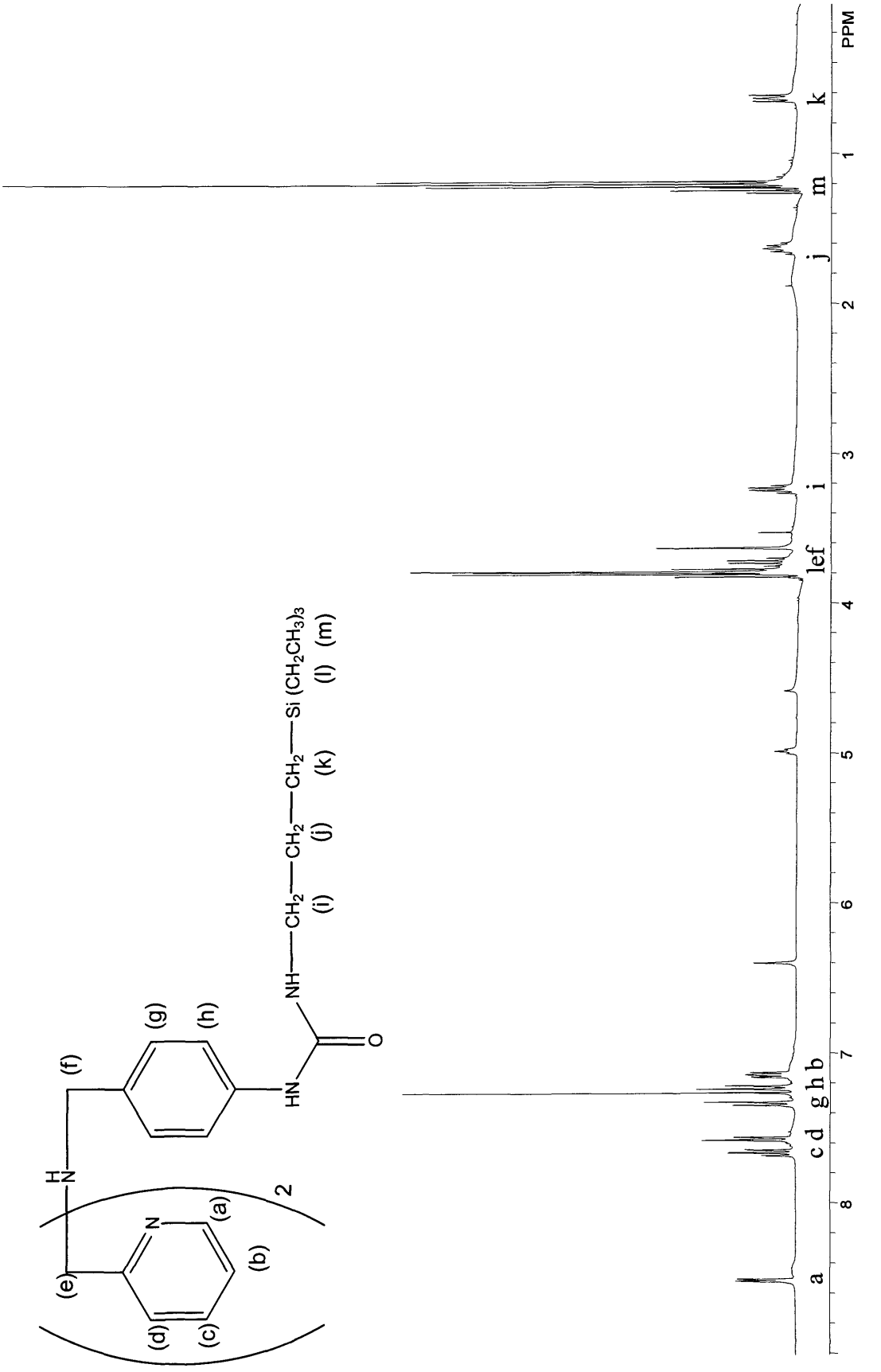
highest activity and of any intermediate produced during DTBC oxidation and H_2O_2 decomposition. In addition, Cu·$BPAS_4$ modified silica catalysts will be evaluated as mimics of dopamine β-monooxygenase and peptidylglycine α-amidating monooxygenase, copper dependent enzymes which have proven to be difficult to model.



Appendix 1: NMR spectrum of $BPAN_4$



Appendix 2: NMR spectrum of $BPAA_4$



Appendix 3: NMR spectrum of $BPAS_4$

References

1. Linder, M.C.; Goode, C. A. *Biochemistry of Copper*, Plenum: New York, 1991.
2. Solomon, E. I.; Sundaram, U. M.; Machonkin, T. E. *Chem. Rev.* **1996**, *96*, 2563-2605.
3. Malmstrom, B. G. *Chem. Rev.* **1990**, *90*, 1247-1260.
4. Farrar, J. A.; Thomson, A. J.; Cheesman, M. R.; Dooley, D. M.; Zumft, W. G. *FEBS Lett.* **1991**, *294*, 11-15.
5. Karlin, K. D.; Tyeklar, Z. *Bioinorganic Chemistry of Copper*, Chapman & Hill: New York, 1993.
6. Themolieres, J. B.; Bieth, J. B. *Phytochemistry* **1984**, *23*, 501-505.
7. Gerdemann, C.; Eicken, C.; Krebs, B. *Acc. Chem. Res.* **2002**, *35*, 183-191.
8. Van Gelder, C. W.; Flurkey, W. H.; Wichers, H. J. *Phytochemistry* **1997**, *45*, 1309-1323.
9. Klabunde, T.; Eicken, C.; Sacchettini, J. C.; Krebs, B. *Nat. Struc. Biol.* **1998**, *5*, 1084-1090.
10. Eicken, C.; Zippel, F.; Buldt-Karentzopoulos, K.; Krebs, B. *FEBS Lett.* **1998**, *436*, 293-299.
11. Grinstead, R. R. *Biochemistry.* **1964**, *3*, 1308-1314.
12. Tsuji, J.; Takayanagi, H. *J. Am. Chem. Soc.* **1974**, *96*, 7349-7350.
13. Lever, A. B. P.; Ramaswamy, B. S.; Pickens, S. R. *Inorganica Chimica Acta.* **1980**, *46*, L59-L61.
14. Moore, K.; Vigee, A. S. *Inorg. Chim. Acta.* **1982**, *66*, 125-130.

15. U. Casellato, S.; Tamburini, P. A.; Vigato, A. De Stefani. *Inorg. Chim. Acta.* **1983**, *69*, 45-51.

16. Casella, L.; Cargo, O.; Gullotti, M.; Garofani, S.; Zanello, P. *Inorg. Chem.* **1993**, *32*, 2056-2067.

17. Malachowski, M. R.; Dorsey, B.; Sackett, J. G.; Kelly, R. S.; Ferko, A. L.; Hardin, R. N. *Inorg. Chim. Acta.* **1996**, *249*, 85-92.

18. Davies, G.; Higgins, R.; Loose, D. J. *Inorg. Chem.* **1976**, *15*, 700-705.

19. Yermakov, Y. I.; Kuznetsoc, B. N.; Zacharov, V. A. Catalysis by Supported Complexes, Elsevier, Amsterdam, The Netherland, 1981.

20. Watton, S. P.; Taylor, C. M.; Kloster, G. M.; Bowman, S. C. *Prog. Inorg. Chem.* **2003**, *51*, 333-420.

21. Bowman, S. C. Silica supported copper complexes and their biomimetic activities (dissertation). **2002**.

22. Malmstrom, B. G.; Enzymology of oxygen. *Annu. Rev. Biochem.* **1982**, *51*, 21-59.

23. Shepard, W. E. B.; Anderson, B. F.; Lewandoski, G. E.; Norris, D. N.; Baker, J. *J. Am. Chem. Soc.* **1990**, *112*, 7817-7819.

24. Spira-Solomon, D. J.; Allendorf, M. D.; Solomon, E. I. *J. Am. Chem. Soc.* **1986**, *108*, 5318-5328.

25. Allendorf, M. D.; Spira, D. J.; Solomon, E. I. *Proc. Natl. Acad. Sci. U. S. A.* **1985**, *82*, 3063-3067.

26. Messerschmidt, A.; Ladenstein, R.; Huber, R.; Bolognesi, M.; Avigliano, L.; Petruzzelli, R.; Rossi, A.; Finazzi-Agro, A. *J Mol. Biol.* **1992**, *224*, 179- 205.

27. Lippars, S. J.; Berg, J. M.; *Principle of Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, 1994.

28. Antholine, W. E.; Kastrau, D. H. W.; Steffens, G. C. M.; Buse, G.; Zumft, W. G.; Kroneck, P. M. H. *Eur. J. Biochem.* **1992**, *209*, 875-881.

29. Kroneck, P. M. H.; Antholine, W. E.; Koteich, H.; Kastrau, D. H. W.; Neese, F.; Zumft, W. G. In *Bioinorganic Chemistry of Copper*, Karlin, K. D., Tyeklar, Z., Eds.; Chapman & Hall: New York, 1993.

30. Tsukihara, T.; Aoyama, H.; Yamashita, E.; Tomizaki, T.; Yamaguchi, H.; Shinwaza-Itoh, K.; Nakashima, R.; Yaono, R.; Yoshikawa, S. *Science.* **1996**, *272*, 1136-1144.

31. Tsukihara, T.; Aoyama, H.; Yamashita, E.; Tomizaki, T.; Yamaguchi, H.; Shinwaza-Itoh, K.; Nakashima, R.; Yaono, R.; Yoshikawa, S. *Science.* **1995**, *269*, 1069-1074.

32. Iwata, S.; Ostermeier, C.; Ludwig, B.; Michel, H. *Nature.* **1995**, *376*, 660-669.

33. Karlin, K. D.; Gultneh, Y.; Nicholson, T.; Zubieta, J. *Inorg. Chem.* **1985**, *24*, 3725-3727.

34. Pockcliffe, D. A.; Martell, A. E. *Inorg. Chem.* **1993,** *32*, 3143-3152.

35. Monzani, E.; Quinti, L.; Perotti, A.; Casella, L.; Gullotti, M.; Randaccio, L.; Geremia, S.; Nardin, G.; Faleschini, P.; Tabbi, G. *Inorg. Chem.* **1998**, *37*, 553-562.

36. Monzani, E.; Quinti, L.; Perotti, A.; Casella, L.; Gullotti, M.; Santagostini, L.; Nardin, G.; Randaccio, L.; Geremia, S.; Zanello, P.; Opromolla, G. *Inorg. Chem.* **1999**, *38*, 5359-5369.

37. Torelli, S.; Belle, C.; Gautier-Luneau, I.; Pierre, J. L.; Saint-Aman, E.; Latour, J. M.; Le Pape, L.; Luneau, D. *Inorg. Chem.* **2000**, *39*, 3526-3536.

38. Rompel, A.; Fischer, H.; Meiwes, D.; Buildt-Karentzopoulos, K.; Dillinger, R. ; Tuczek, F.; Witzel, H.; Krebs, B. *J. Biol. Inorg. Chem.* **1999**, *4*, 56-63.

39. Hartley, F. R. Supported Metal Complexes, Reidel, Dordrecht, The Netherlands, 1985.

40. Lindau-Shepard, B. A.; Shaffer, J. B. *Free Radic. Biol. Med.* **1993**, *15*, 581-588.

41. Paschke, J. ; Kirsch, M.; Korth, H.; deGroot, H.; Sustmann, R. *J. Am. Chem. Soc.* **2001**, *123*, 11099-11100.

42. Zamocky, M.; Koller, F. Prog. *Biophys. Mol. Biol.* **1999**, *72*, 19-66.

43. Kremer, M. L. *Int. J. Chem. Kinet.* **1985**, *17*, 1299-1314.

44. Sigel, H.; Flierl, C.; Griesser, R. *J. Am. Chem. Soc.* **1969**, *91*, 1061-1064.

45. Luo, Y.; Kustin, K.; Epstein, I. R. *Inorg. Cehm.* **1988**, *27*, 2489-2496.

46. Bonomo, R. P.; Marchell, R.; Tabbi, G. J. *Inorg. Biochem.* **1995**, *60*, 205-218.

47. Da Mota, M. M.; Rodgers, J.; Nelson, Samuel Martin. *J. Chem. Soc A*: *Inorganic, Physical, Theoretical.* **1969**, *13*, 2036-2044.

48. Park, Y. J.; Jung, K. H.; Park, K. K.; Eom, T. Y. *J. Colloid Interfac. Sci.* **1993**, *160*, 324-331.

49. Sandell, E. B. Determination of Traces of Metals, Interscience publishers: New York, 1950.

50. Taylor, C. M.; Watton, S. P.; Bryngelson, P. A.; Maroney, M. J. *Inorg Chem.* **2003**, *42*, 312-320.

51. Louloudi, M.; Deligiannakis, Y.; Hadjiliadis, N. *Inorg. Chem.* **1998**, *37*, 6847-6851.

52. Johnson, G. R. A.; Nazhat, N. B. *J. Am. Chem. Soc.* **1987**, *109*, 1990-1994.

53. Phifer, C. C.; McMillin, D. R. *Inorg. Chem.* **1986**, *25*, 1329-1333.

54. March, J. Advanced Organic Chemistry, John Wiley & Sons, Canada, 1992, 4th, 1216.

55. Ohrlein, R.; Schwab, W.; Ehrler, R.; Jager, V. *Synthesis.* **1986**, 535-538.

56. Nagao, H.; Komeda, N.; Mulaida, M.; Suzuki, M.;Tanaka, K. *Inorg. Chem.* **1996**, *35*, 6809-6815.

57. Courtney, R. C.; Gustafson, R. L.; Chaberek, S. Jr.; Martell, A. E. *J. Am. Chem. Soc.* **1959**, *81*, 519-524

58. Gustafson, R. L.; Martell, A. E. *J. Am. Chem. Soc.* **1959**, *81*, 525-529.

59. Harris, C. M.; Sinn, E.; Walker, W. R.; Woolliams, P. R. *Aust. J. Chem.* **1968**, *21*, 631-640.

60. Jallabert, C.; Lapinte, C.; Riviere, H. *J. Mol. Catal. A.* **1982**, *14*, 75-86.

VITA

Jun Wang

The author was born in Hubei, China, February 21, 1979. She graduated from Wuhan University in Wuhan, Hubei, China, July 1997, B.S.

From August 2001 to August 2003, the author attended the College of William and Mary as a graduate assistant in the department of chemistry. The author will receive her M.S. in chemistry in December 2003. She started doctoral studies in chemistry at Johns Hopkins University in August 2003.